

Review

The Emerging Role of Disturbed CoQ Metabolism in Nonalcoholic Fatty Liver Disease Development and Progression

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Abstract: Although non-alcoholic fatty liver disease (NAFLD), characterised by the accumulation of triacylglycerol in the liver, is the most common liver disorder, the causes of its development and progression to the more serious non-alcoholic steatohepatitis (NASH) remain incompletely understood. Oxidative stress has been implicated as a key factor in both these processes, and mitochondrial dysfunction and inflammation are also believed to play a part. Coenzyme Q (CoQ) is a powerful antioxidant found in all cell membranes which has an essential role in mitochondrial respiration and also has anti-inflammatory properties. NAFLD has been shown to be associated with disturbances in plasma and liver CoQ concentrations, but the relationship between these changes and disease development and progression is not yet clear. Dietary supplementation with CoQ has been found to be hepatoprotective and to reduce oxidative stress and inflammation as well as improving mitochondrial dysfunction, suggesting that it may be beneficial in NAFLD. However, studies using animal models or patients with NAFLD have given inconclusive results. Overall, evidence is now emerging to indicate that disturbances in CoQ metabolism are involved in NAFLD development and progression to NASH, and this highlights the need for further studies with human subjects to fully clarify its role.

Keywords: non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; Coenzyme Q; antioxidant; oxidative stress; inflammation; steatosis

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), a condition in which triacylglycerol (TAG) accumulates in the liver, is the most common liver disorder worldwide [1,2]. Its occurrence in the general population has been estimated to be 20%–30%, but in increasingly prevalent metabolic disorders, including obesity, diabetes type 2 and the metabolic syndrome, this rises to 70%–90%. NAFLD itself is considered to be relatively benign, but in some cases its progression leads to liver conditions such as non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and carcinoma which are more serious and may be life-threatening [1,2]. A better understanding of the causes of the development and progression of the disease, therefore, is now of great importance from a public health perspective [1,2].

NAFLD is known to be a multifactorial disease, but the reasons why progression occurs in some cases but not others are not yet well defined [3,4]. Recent work, however, has indicated that oxidative stress resulting from both insulin resistance and hepatic steatosis is likely to be a major causal factor in the development of NAFLD, and may also play an important part in its progression

biosynthesis such as aprataxin, which has a role in double stranded DNA repair, mitochondrial DNA depletion, or the use of statins to combat hypercholesterolemia [21,24,25]. Although endogenous synthesis is normally able to meet the body's need for CoQ, there are circumstances in which deficiencies not caused by genetic defects may occur. Statins, drugs widely used to lower blood cholesterol, can depress CoQ synthesis as they inhibit 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in the pathway of cholesterol synthesis which includes the formation of the isoprenoid units required to produce CoQ [21,26]. In addition, CoQ levels may be pathologically changed in conditions associated with oxidative stress and in aging [27]. Current evidence suggest that dietary CoQ supplementation may be beneficial in many of these cases, but it is not yet used routinely in clinical practice [24,28].

Antioxidant and Anti-Inflammatory Functions of CoQ

CoQ is biosynthesised in all tissues of the body, and, although it was initially believed to be confined to the inner mitochondrial membrane because of its function in the respiratory chain [29], it is now known to be present in all cell membranes [8,23]. Because of its widespread distribution and membrane location, CoQ is the most highly efficient of the naturally occurring lipophilic antioxidants [8]. In the membrane CoQ is in close proximity to lipid and protein molecules in need of protection from locally produced peroxy radicals. Much of the CoQ found in the body is in the antioxidant quinol form (Figure 1), although this varies between tissues [8]. The quinol prevents lipid peroxidation by inhibiting the initial formation and propagation of lipid peroxy radicals (LOO), and in the process it is oxidised to the quinone (Figure 1) and H_2O_2 is produced. In addition, it has been shown to protect proteins from oxidation by a similar mechanism [30], and to prevent oxidative DNA damage such as strand breakages [8,31]. As well as its role in membranes, CoQ is also believed to function in the blood to protect lipoproteins such as very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins from oxidation [8]. The oxidised quinone product is rapidly and efficiently reduced to the quinol by enzyme systems in both the blood and the tissues, thus maintaining a high level of the active form [8].

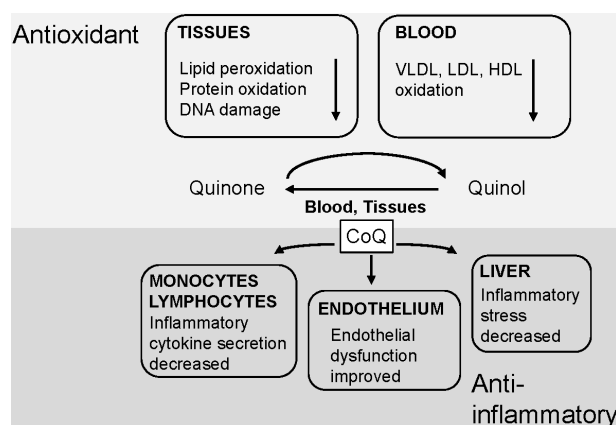


Figure 2. Antioxidant and anti-inflammatory functions of CoQ. Lipids, proteins and DNA and lipoproteins in the blood are protected from oxidative damage by the conversion of the quinone form of CoQ to the quinol form. The quinol is rapidly reconverted to the quinone in both blood and tissues. CoQ has also been shown to decrease cytokine secretion in monocytes and lymphocytes, to improve endothelial function and to reduce hepatic inflammatory stress.

As well as acting as an efficient antioxidant, current evidence suggests that CoQ has a number of independent anti-inflammatory effects [31,32]. It has been shown to reduce the secretion of pro-inflammatory cytokines in monocytes and lymphocytes after an inflammatory stimulus via an influence on the expression of NF- κ B-dependent genes [14,24,33,34]. Furthermore, dietary

supplementation with CoQ₁₀ has been reported to improve endothelial dysfunction in patients with diabetes by up-regulating nitric oxide production (patients received 200 mg CoQ₁₀/day for 12 weeks) [24,35,36], and to decrease the hepatic inflammatory stress caused by obesity in mice (see below) [14].

CoQ therapy has an obvious application in mitochondrial disease [28,37], but its antioxidant and anti-inflammatory effects have led to considerable interest in its possible beneficial effects in other chronic diseases, in particular cardiovascular disease [38,39], but also in neurodegenerative disorders including Parkinson's disease, Huntington's disease and Friedreich's ataxia [38,40–42]. It has also been reported to reduce the risk of pre-eclampsia in pregnancy and to increase sperm quality in infertile men [38,43,44]. The list of conditions influenced by CoQ continues to grow, and below we present evidence from recent studies that suggests that changes in its metabolism may also have a role in NAFLD development and progression. The antioxidant and anti-inflammatory functions of CoQ are summarised in Figure 2.

3. Pathogenesis of NAFLD Development and Progression

NAFLD is defined as the presence of abnormal fat deposition in the liver (steatosis) without inflammation in individuals who do not consume excessive amounts of alcohol, and its occurrence is strongly associated with metabolic disorders such as obesity, diabetes and the metabolic syndrome [1,2]. The simple steatosis may progress to non-alcoholic steatohepatitis (NASH) (about 25% of cases), in which the fat accumulation is accompanied by inflammation and hepatocyte ballooning. Approximately 20% of NASH patients will develop fibrosis and cirrhosis, and in 1% of cases per year cirrhosis progresses to liver carcinoma [2]. The hepatic diseases and main clinical conditions involved in NAFLD development are well known and described in terms diagnostic criteria [45], and, although the pathogenesis from healthy liver to the disease condition remains incompletely understood, oxidative stress and insulin resistance have been strongly implicated as causative factors [5,46].

The finding that the risk of progression of NAFLD to NASH is related to the severity of fat deposition led Day *et al.* [47] to propose the “two hit” hypothesis in 1998. The first hit is the simple hepatic steatosis which results from the development of insulin resistance, and this leads to conditions in which the liver becomes more sensitive to the second hits which include oxidative stress, mitochondrial dysfunction and inflammation [2,4,47]. More recently, however, it has become clear that polymorphism in the gene for patatin-like phospholipase 3 (PNPLA3) also plays a role in NASH development, thus it is more accurately described as a multiple hit process [4].

In the normal liver, there is a balance between the synthesis and esterification of fatty acids (FFA) which increases hepatic TAG content, and VLDL secretion and fatty acid oxidation which decreases it [48]. In insulin resistance, however, there is an increased supply of fatty acids to hepatocytes, causing not only fat accumulation but also a rise in mitochondrial β -oxidation, and consequently increased oxidative stress caused by raised levels of reactive oxygen species (ROS), lipid peroxidation, protein oxidation and the production of pro-inflammatory cytokines [5,49]. Recent studies have also suggested that oxidative stress may be a primary cause of liver fat accumulation [50], and ROS have also been shown to play a part in fibrosis development [51]. In addition, blood antioxidant concentrations have been reported to be decreased in patients with NAFLD [52]. It is now clear, therefore, that oxidative stress is a major factor in the development of NAFLD and its progression to NASH. Furthermore, a large proportion of cellular ROS are produced in mitochondria, and overproduction of ROS when mitochondrial respiration is disrupted has been suggested to be important in NAFLD development [53–55]. The presence of mitochondrial dysfunction in patients with NASH also indicates that it is likely to have important role in the pathogenesis of the disease [56,57]. Figure 3 summarises current ideas on the pathogenesis of NAFLD development and progression.

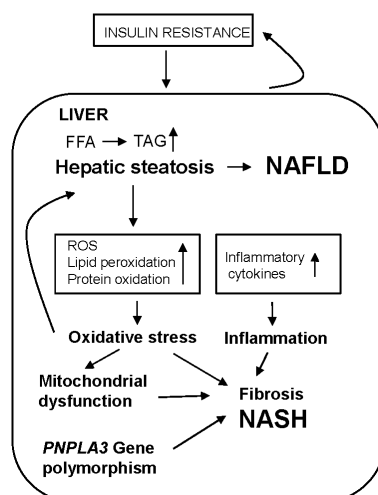


Figure 3. The development and progression of NAFLD. The development of insulin resistance increases the supply of free fatty acids (FFA) to the liver causing increased TAG synthesis and hepatic steatosis. Oxidative stress may also contribute to this process. The steatosis leads to inflammation and increased oxidative stress, which in turn causes mitochondrial dysfunction. These conditions, together with other factors such as *PNPLA3* gene polymorphism, result in the development of fibrosis and ultimately NASH. In the traditional 2 hit hypothesis [48] the initial steatosis is the first hit and the second hits are the resulting oxidative stress and inflammation. It is now clear, however, that multiple hits are involved in the process.

4. CoQ and NAFLD Development and Progression

Both the role of CoQ in mitochondria and its function as an efficient endogenously synthesised antioxidant [8,10,21] suggest that it may play a part in the development of NAFLD and its progression to NASH, and evidence for this has begun to emerge from recent investigations. In general, two main approaches have been used. The first has been to study the relationship between the disease and CoQ metabolism in the body and the second to assess the influence of dietary supplementation with CoQ on the development of the condition. So far, the majority of studies have used animal models of NAFLD or NASH, mainly in rats and mice, but as interest grows, more human studies are being undertaken.

Studies to date on how CoQ metabolism is changed in NAFLD have given conflicting results. As early as 1996, Eaton *et al.* [18] suggested from their work on β -oxidation in patients with alcoholic and non-alcoholic liver disease that decreased amounts of CoQ may contribute to hepatic steatosis in NASH, and more recently, Yesilova *et al.* [19] found that CoQ₁₀ levels were depleted in men with NAFLD in comparison with normal subjects. Moreover, these authors also showed that there was a negative correlation between CoQ₁₀ and body fat and also inflammatory activity and fibrosis [19]. In an investigation with obese rats, exposure of the animals to a mixture of environmental contaminants implicated in NAFLD development and progression has been reported to cause a decrease in liver CoQ₁₀ concentrations [57], and the activity of geranylgeranyl pyrophosphate synthase, an enzyme involved in the formation of isoprene units, was also decreased in these conditions. The authors conclude from this that *de novo* synthesis of CoQ is depressed in NAFLD, but measurement of levels of CoQ₉, the main endogenous form in rats, would be required to confirm this. In contrast, Bravo *et al.* [20] found that plasma levels of CoQ₉ were increased in rats with high fat diet-induced NAFLD, while concentrations of CoQ₁₀, which is likely to be mainly derived from exogenous sources, were not significantly changed. Furthermore, there was an increase of about 75% in the proportion of total plasma CoQ (CoQ₉ + CoQ₁₀) in the reduced form. Another study using a mouse model of NASH found increased levels of plasma CoQ₉ in the animals, and the rise was positively correlated with hepatic ROS concentrations, hypercholesterolemia and fibrosis [58]. Since increases were observed in CoQ₉, but not CoQ₁₀ in these experiments [20,58], it seems likely that this change reflects

up-regulation of endogenous synthesis which may be a protective response against a general reduction in the antioxidant capacity of the plasma which occurs in NAFLD [15,52]. In addition, respiratory activity and the activity of liver mitochondrial NADH-CoQ oxidoreductase (complex I of the respiratory chain) have been shown to be decreased in rats with NAFLD induced by choline deficiency [9]. Current evidence, therefore, clearly indicates that CoQ metabolism is disturbed in NAFLD, but further studies are required to elucidate how exactly this relates to the development and progression of the disease.

The finding that changes in CoQ metabolism are potentially involved in the development and progression of NAFLD raises the question of whether dietary supplementation with the quinone may be beneficial in the prevention or treatment of the condition. Although only a few studies have investigated the direct effects of CoQ supplementation on NAFLD (see below), a number of investigations in both humans and animals have provided evidence in areas known to be associated with the disease such as oxidative stress and mitochondrial dysfunction (studies on dietary supplementation with CoQ are summarised in Table 1). Despite the fact that CoQ₉ is the endogenous form in rats and mice, with just a few exceptions (see below), investigations with animals have used CoQ₁₀ as the dietary supplement.

Dietary supplementation with CoQ₁₀ (300 mg/day for 12 days) has been found to reduce serum markers of oxidative stress in young swimmers [59] and, in a series of studies in patients with coronary artery disease (60–300 mg CoQ₁₀/day for 12 weeks), to reduce oxidative stress and increase the activity of antioxidant enzymes as well as lowering inflammation as assessed by plasma levels of inflammatory markers such as tumour necrosis factor (TNF)- α and interleukin (IL)-6 [12,13,60,61]. Similarly, dietary CoQ (diet supplemented with 0.07%–0.7% (w/w) CoQ₁₀ for 26 weeks) was associated with a reduction in plasma oxidative stress and inflammatory markers in a rat model of the metabolic syndrome [62]. In healthy sedentary men given oral CoQ₁₀ (100 mg/day for 8 weeks), however, Gobkel *et al.* [11] reported that blood levels of TNF- α and interleukin (IL)-6 were unchanged over the 8 week experimental period.

In the liver in general, animal studies have shown that dietary CoQ₁₀ is hepatoprotective against toxic agents including acetaminophen (rats were given a single oral dose of acetaminophen (700 mg/kg), two injections (i.p.) of CoQ₁₀ at 1 and 12 h and killed at 24 h) and carbon tetrachloride (a single i.p. dose of 200 mg/kg CoQ₁₀ was administered 24 h before injection of CCl₄ (1 mL/kg) s.c. and animals were killed 24 h later) in rats [63,64], inhibits liver fibrosis induced by dimethylnitrosamine (DMN) (10 or 30 mg CoQ₁₀ given orally before administration of mDMN and 3 times/week thereafter for 3 weeks) or eggs of the parasitic worm *Schistoma mansoni* (*S. mansoni*) (oral dose of 5 mg/kg CoQ₁₀ daily for 8 or 12 weeks after infection) in mice [65,66], and limits ischemic damage in transplanted livers when the donor rat is pretreated (10 mg/kg CoQ₁₀ given i.v. 1 h before induction of ischemia) [67]. Intraperitoneal administration of CoQ₁₀ (8 mg i.p. every second day for 38 days) has been shown to lower liver TAG content in rats [68], suggesting that it could be protective against hepatic fat deposition in NAFLD. Hepatic oxidative stress and inflammation has also been found to be reduced by CoQ₁₀ therapy in several animal studies. In a model of diet-induced obesity, a high risk condition for NAFLD development in mice, Sohet *et al.* [14] showed that the expression of mRNA for a panel of hepatic genes involved in ROS production, inflammation and metabolic stress was reduced in the animals fed CoQ (diet supplemented with 1% CoQ₁₀ for 60 days), and Othman *et al.* [66], also found that the quinone decreased oxidative stress and preserved antioxidant factors in livers of mice infected with *S. mansoni* (study details given above). In contrast, in another study although both CoQ₉ and CoQ₁₀ levels in the liver were increased in rats fed a diet supplemented with CoQ₁₀ (62 mg/kg) for 6 or 12 months, the activities of the hepatic antioxidant enzymes measured were either unchanged or decreased [69]. Hepatic mitochondrial dysfunction has also been shown to be improved by CoQ₁₀ (0.57 mg/day for 6 weeks) in rats given a high cholesterol diet and atorvastatin [70] and in cultured human hepatocytes treated with D-galactosamine, an inducer of oxidative stress and cell death and 30 μ M CoQ [71].

Table 1. Summary of studies on the effects of dietary CoQ supplementation on NAFLD and related factors.

Subjects/Animals/Cells	Oxidative Stress (OS)	Inflammation	Other	References
Young swimmers	Plasma OS markers MDA, NO, protein hydroperoxide decreased; total antioxidant capacity increased			[59]
Patients with coronary artery disease	Plasma MDA reduced; SOD, CAT, GPx activities increased	Plasma TNF- α , IL-6 reduced		[12,13,60,61]
Rats with the metabolic syndrome	Plasma oxidised LDL decreased	Plasma hsCRP reduced	Endothelial dysfunction improved	[62]
Healthy sedentary men		Plasma TNF- α , IL-6 levels unchanged		[11]
Rats with liver injury induced by acetaminophen or CCl ₄	Liver GSH increased; lipid peroxides decreased		Liver tissue damage ameliorated; liver NF- κ B, caspase 3 and inducible NO synthase	[63,64]
Mice with diet-induced obesity	Liver expression of NADPH oxidase decreased	Liver expression of CRP, STAMP2 decreased	Liver expression of CPT1 α decreased	[14]
Mice with liver fibrosis induced by DMN or <i>S mansoni</i>	Liver MDA decreased; GSH increased	Liver TGF- β reduced, Nrf2/ARE activated	Liver fibrosis decreased	[65,66]
Liver transplantation donor rats			Ischemic damage in transplanted liver prevented	[67]
Rats (CoQ ₁₀ given IP)			Lipid content of liver decreased	[68]
Rats fed sunflower oil or olive oil	Liver antioxidant enzyme activities unchanged or decreased			[69]
Rats fed a high cholesterol diet and atorvastatin			Serum and liver cholesterol and TG lowered; mitochondrial respiration improved	[70]
Rats with diet-induced NAFLD *	Plasma oxidised CoQ ₉ increased		Liver injury, steatosis, VLDL production unchanged; microsomal apoB, TG and membrane phospholipid increased; plasma leptin increased	[15,16]
Cultured human hepatocytes treated with D-galactosamine	ROS generation decreased		Electron transport chain dysfunction improved	[71]
Humans with NAFLD	Serum total antioxidant capacity decreased		Serum AST decreased, waist circumference decreased	[46]
Humans with NAFLD	Oxidative stress status unchanged		No beneficial effects on serum lipid profile or blood pressure	[72]

ARE: antioxidant response element; AST: aspartate aminotransferase; CAT: catalase; CPT1 α : carnitine palmitoyl transferase 1 α ; CRP: C-reactive protein; DMN: dimethylnitrosamine; GPx: glutathione peroxidase; GSH: reduced glutathione; hsCRP: high sensitivity CRP; MDA: malondialdehyde; SOD: superoxide dismutase; STAMP2: six transmembrane protein of prostate 2. *CoQ₉ was used as the dietary supplement. In all other studies listed CoQ₁₀ was used.

The studies discussed above suggest that CoQ therapy may be beneficial effect in alleviating some of the causal factors of NAFLD, including oxidative stress, inflammation and mitochondrial dysfunction. Building on this base, our group investigated the direct effects of dietary supplementation with CoQ₉ on the development of the disease using a high fat diet- induced model in rats [15,16]. CoQ₉ was used rather than CoQ₁₀ as it is the main endogenously synthesised form in the test species [21]. We used a dose of 30 mg/kg/day CoQ₉ for 8 weeks after feeding a high fat diet for 10 weeks. NAFLD was found to be associated with increased, rather than reduced, plasma CoQ₉ levels, while CoQ₁₀ (mostly originating from the diet) concentrations were unaffected. However, the ratio of CoQ₉:CoQ₁₀ was significantly higher in NAFLD rats given the dietary supplement and plasma oxidized CoQ₉ levels were also raised, effects which may be responses to increased oxidative stress [15]. Dietary CoQ₉ was not found to have significant effects on hepatic steatosis, liver injury, the development of insulin resistance or increased production of VLDL seen in NAFLD, although it did cause a rise in plasma leptin levels, an effect which is likely to be detrimental rather than beneficial.

Under normal physiological conditions, the liver produces and secretes VLDL when energy for peripheral tissues is needed. TAG is transferred into the endoplasmic reticulum (ER), where it enters the VLDL precursor pathway [73]. In NAFLD, however, there is overproduction of large, TAG-rich VLDL causing hypertriglyceridemia [74]. Since a number of crucial steps in VLDL synthesis depend on the proper functioning of the ER membrane [73], Cano and colleagues carried out another study to investigate the effects of dietary CoQ₉ on hepatic VLDL processing in rats with NAFLD [16]. CoQ₉ was shown to have a number of effects including increasing microsomal levels of apoB and TAG and changing the microsomal membrane phospholipid content [16]. This kind of membrane change could delay the transfer of the nascent lipoproteins out of the ER into the secretory pathway, triggering increased apoB synthesis and TAG-rich VLDL particle size, and leading to the secretion of larger particles while the total concentration of VLDL TAG remains unchanged. Thus, although dietary CoQ₉ was shown to affect VLDL secretion in hepatic steatosis, the changes may not be beneficial as they could lead to the production of larger, more atherogenic VLDL.

In human subjects with NAFLD, oral CoQ₁₀ (100 mg/day for 4 weeks) has been reported to reduce waist circumference and decrease serum levels of aspartate aminotransferase, but was also found to decrease serum total antioxidant capacity [46], and in a second study (100 mg daily for 12 weeks), no beneficial effects were found [72]. Clearly, in considering dietary supplementation with CoQ as a therapy for NAFLD, it is important to establish whether it is able to bring about a reduction in hepatic fat accumulation and to prevent the development of fibrosis and carcinoma in the longer term. Because of the limited number of studies which have investigated effects on NAFLD directly, however, conclusive evidence is not yet available in these areas. CoQ administration has been reported to decrease hepatic steatosis in rats in one study [68], but in our work using a rat model of NAFLD we found no change [15]; liver fibrosis and hepatocellular carcinoma have been shown to be inhibited in rodents [65,66,75], but in these cases the conditions were induced chemically rather than as a result of NAFLD/NASH. Overall, therefore, studies to date, have not shown any direct benefit of CoQ therapy in combatting NAFLD development, however, as they are so few there is clearly a need for further investigations, and in particular for more human intervention studies, before any definitive conclusions can be reached.

5. Summary and Conclusions

Recent research has shown that oxidative stress, mitochondrial dysfunction and inflammation are key factors in the development of NAFLD and its progression to NASH [2,4,47]. The essential role of CoQ in mitochondria, therefore, together with its function as an efficient, endogenously synthesised antioxidant and its anti-inflammatory properties [8,10,21,24] suggest that disturbances in its metabolism may be involved in the pathogenesis of the disease, and evidence for this is now emerging. Investigations, mainly using animal models so far, have shown that CoQ metabolism is altered as NAFLD progresses, although its exact relationship to the disease process remains

to be determined. Experiments with both animals and humans have demonstrated that dietary supplementation with CoQ is potentially beneficial for NAFLD, since it reduces oxidative stress and inflammation, improves mitochondrial dysfunction and is generally hepatoprotective. However, only a few studies either in animal models or in patients have assessed the direct effects of dietary CoQ in NAFLD to date, and the results have been inconclusive. Despite this, current evidence clearly indicates that CoQ is likely to be involved in NAFLD development and further studies, particularly with human subjects, are needed both to fully elucidate its role and to resolve the question of the efficacy of CoQ therapy in alleviating the effects of the condition.

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Conflicts of Interest: The authors declare no conflict of interest.

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