Effect of Citicoline on liver fat content in mice fed a high-fat diet, an experimental study

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Abstract

Citicoline may have a beneficial role in the protection of hepatocytes because of its role in reducing oxidative stress and its choline part role in the production of VLDL. Choline deficiency leads to liver fat accumulation, which is treated by supplying choline. The purpose of this study was to investigate the hypothesis, of whether the administration of citicoline, the precursor of choline, in mice on a high-fat diet is effective in reducing the amounts of liver fat content.

Twenty-four male C57BL/6 mice, after 2 weeks on a high-fat (HF) diet, were randomly assigned to the 4 groups. Control: HF diet mice without intervention, dose 1: HF diet mice with a dose of 10 mg/kg Citicoline, dose 2: HF diet mice with an amount of 20 mg/kg citicoline, and dose 3: HF diet mice with an amount of 30 mg/kg Citicoline.

After 8 weeks, the mice's body weights, liver weights, liver dry weights, and liver fat percentages were measured. All the aspects of ARRIVE and PREPARE guidelines were considered.

The differences in liver fat % between the control, dose 1, and dose 2 groups were not statistically meaningful (8.37 ± 0.39, 8.08 ± 0.65, 7.63 ± 0.55, and 7.34 ± 0.65 % for control, dose 1, dose 2, and dose 3 groups respectively; p for ANOVA test: 0.0381). A posthoc Tukey test showed that liver fat % was statistically different only between the control and dose 3 groups (mean diff: 1.03, 95%CI: 0.05 to 2.0).

The liver total weight and liver dry weight were not different between the groups (p= 0.94, and p= 0.66 respectively). The intervention did not affect the mice's body weights.

It seems that citicoline can be considered as a dietary supplement in the treatment of fatty liver. RCT studies are suggested to test this hypothesis.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a general term for the histological spectrum of a group of diseases from hepatic steatosis to nonalcoholic steatohepatitis (NASH), an inflammatory phenotype with liver damage, with or without fibrosis, that can progress to cirrhosis with complications of liver failure and liver cancer. Hepatic steatosis occurs when FA entry, through dietary fat (especially saturated fatty acids) or endogenous hepatic lipogenesis, or TG synthesis from diacylglycerol exceeds lipids exit from the liver or oxidation (1, 2). NAFLD affects about 30% of the general population worldwide (3). Conventional interventions, such as lifestyle modification, regular exercise, and a healthy diet, have been effective in improving non-alcoholic fatty liver disease by reducing liver fat (4). Currently, there are no drugs approved by the FDA for the treatment of NAFLD worldwide (4, 5).

Citicoline is an endogenous chemical compound which is also available as a dietary supplement. It stands for cytidine-5’-diphosphocholine (CDP-choline). In the human body, citicoline is broken down into cytidine and choline during hydrolysis and dephosphorylation. Cytidine and choline are substrates for the
synthesis of phosphatidylcholine and CDP-choline in neurons (6). It has minimal toxicity and is rapidly metabolized. Metabolic products are removed as carbon dioxide. The safety of citicoline has been repeatedly proven in animal studies (7).

Citicoline has been shown that has neuroprotective properties (6). It may also have a beneficial effect on neurodegenerative diseases such as Parkinson's and Alzheimer's disease (8, 9).

Citicoline may also have a beneficial role in the protection of hepatocytes (10, 11). A proposed mechanism for hepatoprotection is its role in the reduction of oxidative stress (11).

Citicoline is a source of choline and cytidine. Three health claims are allowed for choline which is related to the beneficial effects of a nutrient on certain normal functions of the body. The first two claims state that choline contributes to normal fat metabolism and maintenance of normal liver function. These claims were accepted because they were supported by a body of observations that choline deficiency was associated with signs of liver damage including the development of fatty liver in humans fed with choline-free parenteral nutrition solutions, which were reversed by dietary choline administration (12, 13). The third claim, which states that choline contributes to the normal metabolism of homocysteine, is supported by the observation that choline-deficient diets tend to increase plasma homocysteine concentrations (14).

Choline is a nutrient that is obtained through dietary intake and endogenous synthesis (15). Foods high in choline include dairy products, liver, eggs, legumes, nuts, beef, leafy vegetables, oilseeds, and grain sprouts (15). Choline has several important functions in the human body. It is a source of methyl groups needed to make the primary methyl donor S-adenosylmethionine. It is part of the neurotransmitter acetylcholine. It is also a component of the major phospholipids in membranes, phosphatidylcholine (PC) or Lecetin and sphingomyelin. PC is one of the main components of VLDLs and is required for their secretion and lipid removal from the liver. The increase in TG synthesis may be due to the decrease in phosphatidylcholine concentration (16).

Choline is also an important part of the mitochondrial membrane. Mitochondrial dysfunction is one of the main mechanisms in the pathogenesis of NAFLD (17).

Fatty liver occurs in people who are deficient in choline, but it is treated by supplying choline. The purpose of this study was to investigate the hypothesis, of whether the administration of citicoline, the precursor of choline, in mice on a high-fat diet is effective in reducing the amounts of liver fat content.

**Methods & materials**

Twenty-four male C57BL/6 mice, after 2 weeks on a high-fat (HF) diet were randomly assigned to the following 4 groups and kept in separate cages as follows:

Control: HF diet mice without intervention,
Dose 1: HF diet mice with a dose of 10 mg/kg Citicoline,

Dose 2: HF diet mice with an amount of 20 mg/kg Citicoline,

Dose 3: HF diet mice with an amount of 30 mg/kg Citicoline.

Different doses of citicoline were delivered via drinking water. The average daily water consumption of mice has been estimated at 5.8 ± 0.2 ml/mouse by Bachmanov et al (18). We measured 3-day drinking water to estimate the average ad libitum water drinking in the mice in this study. The result was about 5 ml/mouse. The mean mice weight was 20 grams (rounded). Therefore, 3 different concentrations of citicoline in tap water were made every day based on the average body weight and water intake (40 mg/L, 80 mg/L, and 120 mg/L for doses 1, 2, and 3 respectively). Citicoline sodium is a sensitive molecule in acidic, oxidative, and alkaline conditions, but it is stable against light and dry heat (19), and it seems also stable for 8 days at 4 °C and room temperature (20). However, the mice's water bottles were covered with foil to avoid the possible effect of light. During this period, suitable conditions were provided for mice according to the protocol regarding temperature, light, and nutrition (21). At the end of 8 weeks of intervention, the mice were first anesthetized with Ketamine/Xylazine 80/10 mg/kg IP, and then the animal's weight was measured with a scale with an accuracy of 1 mg. The animals were then beheaded, and all extractable blood was collected, and the blood serum was isolated for further studies. Then the whole liver tissue was removed using surgical instruments. The remains of the animal were exterminated according to the protocols (21).

For each mouse, liver weight was measured using a scale with an accuracy of 1 mg. Then, the entire liver was homogenized in a crucible and moved to a filter paper, and re-weighed.

To calculate the dry weight, the liver sample with filter paper was placed in an oven at 105 degrees Celsius for 3 hours. Then, after cooling, the dried liver was weighed and its amount was deducted from the initial weight of the liver and filter paper, and the remainder was divided by the initial weight of the liver and multiplied by 100. Then, the liver fat extraction process began with a six-cell fully automatic Soxhlet (SOXTHERM®) using ethyl ether as solvent.

In the end, the remaining ethyl ether was removed from the sample by putting it in 105 degrees Celsius for 1 hour where the last 2 weights in 10 minutes were identical. Then, the filter paper containing the liver was weighed again, and its weight was deducted from the weight of the dried liver and filter paper before fat separation (which yields liver fat weight). To calculate the liver fat percentage, the result was divided by the initial weight of the liver and multiplied by 100.

Ethics: In this experimental study, all the aspects of ARRIVE and PREPARE guidelines were considered, and All ethical considerations (21) were followed. This study was performed after approval by the ethics committee of Golestan University of Medical Sciences (ethics code: IR.GOUMS.REC.1399.022, approval date: May 3, 2020). All methods were performed in accordance with the relevant guidelines and regulations.
Results

Through the experiment, 3 mice died in the control, dose 1, and dose 2 groups. Therefore, data were available for 5, 5, 5, and 6 mice in the control, dose 1, dose 2, and dose 3 groups respectively.

The differences in liver fat % between the control, dose 1, and dose 2 groups were not statistically meaningful (8.37 ± 0.39, 8.08 ± 0.65, 7.63 ± 0.55, and 7.34 ± 0.65 % for control, dose 1, dose 2, and dose 3 groups respectively; p for ANOVA test: 0.0381). Post-hoc Tukey test showed that liver fat % was statistically different only between the control and dose 3 groups (mean diff: 1.03, 95%CI: 0.05 to 2.0) (Figure 1).

The liver total weight (Figure 2) and liver dry weight (Figure 3) were not different between the groups (p= 0.94, and p= 0.66 respectively).

The intervention did not affect the mice’s body weight (23.28 ± 1.91, 22.38 ± 1.94, 22.08 ± 3.65, and 22.50 ± 3.47 gr for control, dose 1, dose 2, and dose 3 groups respectively, p= 0.94). It means that no statistical differences were found between the groups in terms of body weight (Figure 4).

Discussion

In this study citicoline in the dose of 30 mg/kg led to a reduction of liver fat in C57BL/6 male mice after 8 weeks on a high-fat diet. Meanwhile, citicoline had no significant effect on body weight, liver weight, and liver dry weight.

Since the mice feeding was ad libitum, no change in body weight shows that citicoline did not affect their appetite and food intake. Therefore, it can be concluded that citicoline probably affects the amount of liver fat through mechanisms independent of caloric intake.

Citicoline is a water-soluble compound, and its optimal absorption is taken orally, which is used commercially in different formulations for pharmacokinetic and clinical purposes (7). In the body, citicoline is rapidly hydrolyzed and converted into choline and cytidine (22). The citicoline pathway includes choline transport, choline kinase, phosphocholine cytidyltransferase, and choline phosphotransferase (22). On a molar mass basis, citicoline is significantly less toxic than choline (23).

The reason for the lower toxicity is that compared to phosphatidylcholine and other choline derivatives found in food, citicoline undergoes less enzymatic hydrolysis in the intestinal lumen. (24). It is important to consider that the gut microbiome metabolizes a significant portion of choline and its derivatives to trimethylamine (TMA), a gaseous metabolite that is readily absorbed and oxidized to Trimethylamine N-oxide (TMAO) in the liver. TMAO is involved in the etiology of many diseases including kidney failure, diabetes, and cancer (23, 25-28).

Choline is a precursor of the neurotransmitter acetylcholine and is also involved in methyl group metabolism, especially in the liver (29, 30). It is also an important component in the structure of
phosphatidylcholine, which plays an essential role in the synthesis of lipoproteins. The ability of the liver to synthesize VLDL is essential to prevent fat accumulation in the liver (30). In the non-fatty liver, there is a homeostatic balance between TG synthesis and excretion. Given that phosphatidylcholine is required for VLDL synthesis/secretion, part of the pathogenesis of NAFLD is expected to be related to liver choline depletion (31). Several studies have shown the effectiveness of betaine on fatty liver (32-35). Although choline can also be affected by the betaine pathway because it can be converted into betaine by the oxidation of choline, it can also have a positive effect on increasing the production of VLDL and helping to exporting TG from the liver through the phosphatidylcholine pathway.

Citicoline has also been shown to attenuate hepatotoxicity in mice by overexpressing Vesicle-associated membrane protein 2 (VAMP2), peroxisome proliferator-activated receptor gamma (PPAR-γ), and sirtuin 3 (SIRT3). SIRT3 has a protective role in improving mitochondrial dysfunction and therefore protection against fat accumulation in the liver and hepatocyte lipotoxicity (36, 37). SIRT3 deficiency has been shown to exacerbate hepatic steatosis by attenuating the HIF-1-dependent lipin1 pathway and increasing CD36 via nuclear factor erythroid 2–related factor 2 (Nrf2) (38).

Based on the findings of this study and considering the lower toxicity of citicoline compared to other choline-containing molecules, it seems that citicoline can be considered as a dietary supplement in the treatment of fatty liver. RCT studies are suggested to test this hypothesis.

Declarations

Ethics approval and consent to participate:

This study was performed after approval by the ethics committee of Golestan University of Medical Sciences (ethics code: IR.GOUUMS.REC.1399.022, approval date: May 3, 2020). All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication:

Not applicable.

Availability of data and materials:

The data that support the findings of this study are available on reasonable request from the corresponding author.

Competing interests:

None.

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**Authors' contributions:**

A S: Literature search; the conception and design of the study; carrying out the study; Acquisition, statistical analysis, and interpretation of the data; drafting the manuscript; final approval of the version to be submitted; agreement to be accountable for all aspects of the work.

Z J: design of the study; interpretation of the data; revision of the manuscript; final approval of the version to be submitted; agreement to be accountable for all aspects of the work.

Sh Gh: design of the study; interpretation of the data; revising the manuscript critically for important intellectual content; final approval of the version to be submitted; agreement to be accountable for all aspects of the work.

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Figure 1

Comparison of liver fat percent between the groups (Control: 5 mice on high-fat diet; dose 1: 5 mice on high-fat diet receiving daily 10 mg/kg citicoline in drinking water; dose 2: 5 mice on high-fat diet receiving daily 20 mg/kg citicoline in drinking water; dose 3: 6 mice on high-fat diet receiving daily 30 mg/kg citicoline in drinking water).
Figure 2

Comparison of liver weight (mg) between the groups (Control: 5 mice on the high-fat diet; dose 1: 5 mice on high-fat diet receiving daily 10 mg/kg citicoline in drinking water; dose 2: 5 mice on high-fat diet receiving daily 20 mg/kg citicoline in drinking water; dose 3: 6 mice on high-fat diet receiving daily 30 mg/kg citicoline in drinking water).
Figure 3

Comparison of liver dry weight (mg) between the groups (Control: 5 mice on high-fat diet; dose 1: 5 mice on high-fat diet receiving daily 10 mg/kg citicoline in drinking water; dose 2: 5 mice on high-fat diet receiving daily 20 mg/kg citicoline in drinking water; dose 3: 6 mice on high-fat diet receiving daily 30 mg/kg citicoline in drinking water).
Comparison of body weight (gr) between the groups (Control: 5 mice on high-fat diet; dose 1: 5 mice on high-fat diet receiving daily 10 mg/kg citicoline in drinking water; dose 2: 5 mice on high-fat diet receiving daily 20 mg/kg citicoline in drinking water; dose 3: 6 mice on high-fat diet receiving daily 30 mg/kg citicoline in drinking water).