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Original Research

Adverse Effects of Long-Term Ice-Cold Water Drink on Rat Liver Function and Histology

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Abstract

Drinking ice-cold water is prohibited in Avicenna's "The Canon of Medicine" book in which he emphasized that ice-cold water drinking was hazardous for the body organs such as the liver. Little information can be found regarding the effects of ice-cold fluid drinks on liver and its probable sequels on this vital organ. Accordingly, we investigated the effects of long-term ice-cold water drink on the rat liver function and histology. Eighteen male Wistar rats, weighing 180 ± 20 g, were randomly divided into three groups of six as two months ice-cold water drink, CW2M; three months ice-cold water drink, CW3M; and three months room temperature water drink; control group. Upon completion of the care period, a blood sample has been taken for liver enzymes and lipid profile assessment. Liver tissue has also been used for histological studies of H&E staining and microscopic examination. Histological findings showed hepatocellular micro-vesicle formation, necrosis and derangement of the cellular cords and infiltration of Kupffer cells in ice-cold water taken animals. Serum TGs, VLDL-C and ALP significantly increased with sound decrease in FBS and LDL-C in ice-cold water taken animals. It seems that long-term ice-cold water has deleterious functional and structural effects on the liver.

Keywords: Lifestyle; Ice-cold water; Liver; Non-alcoholic fatty liver disease (NAFLD); Lipid level; Lipoproteins

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Introduction

Drinking ice-cold water is prohibited in Avicenna's "The Canon of Medicine" book in which he emphasized that ice-cold water drinking was hazardous for the body organs such as liver, bone, brain, stomach, lungs and nerves. He also noted gradual adverse changes followed by ice-cold water drink [1-2].

However, there is a lack of evidence on this impression. A literature search found nothing more than a few scattered articles examining the acute effects of ice-cold water and beverages on certain biological parameters. Drinking ice-cold water is reported to acutely trigger asthma attack [3] and headache [4], alter thermogenesis [5] and energy expenditure [6], blood pressure [7] and heart rate [8], gastrointestinal motility [9-11], intragastric pressure [12], rectal temperature [13,14], and endocrine secretions [15]. On the other hand, solid associations have been found between lifestyle of food taking and development of non-communicable diseases such as metabolic syndrome [16,17]. Non-alcoholic fatty liver disease (NAFLD), a global health concern, is mainly affected by lifestyle misbehavior [18] and may eventually provoke to fibrosis, cirrhosis and hepatocellular carcinoma with weighty health and economic consequences to the patients, their families and the society [19]. Its pathophysiology is multifactorial [20] and influenced by lifestyle [21] as well, especially eating behavior.

To the best of our knowledge, little information can be found regarding the effects of ice-cold fluid drinks, an aspect of eating behavior, on liver and its probable sequels on this vital organ. Accordingly, we investigated the effects of long-term ice-cold water drink on the rat liver function and histology as a preliminary work.

Methods

Animals

Eighteen male Wistar rats, weighing 180 ± 20 g were kept at controlled conditions of 22 ± 2 °C and 12:12-hour light-dark cycle. Animals were randomly divided into 3 groups of 6 rats each. The first group allowed to freely drink ice-cold water (4 °C) for two months (CW2M). The second group did the same for three months (CW3M). The third group took room temperature water for 3 months and were considered as control group. Animals were handled in accordance with the criteria of the 'Guide for the Care and Use of Laboratory Animals', 8th edition (https://www.ncbi.nlm.nih.gov/books/NBK54050/). All experimental procedures were approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1379.802). Ice-cold water was prepared by filling the bottle of water with ice cubes twice a day. At the end of 2 or 3 months, animals were anesthetized with a mixture of intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg) after a twelve hour overnight fast. Four to five ml of blood was taken directly from the heart. The blood was centrifuged, serum separated and stored at -70 °C for further analysis.

The liver was separated from the surrounding tissues and washed with normal saline. Left medial lobe was removed and put in 10% formalin for histological examination.

Biochemical analysis

Serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), Triglycerides (TGs), Total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C) and Fasting blood sugar (FBS) were measured using colorimetric AutoAnalyzer (Roche-Hitachi 902 AutoAnalyzer- Japan, Pars Azmoon Kit). Low-density lipoprotein cholesterol (LDL-C) and Very low-density lipoprotein cholesterol (VLDL-C) were calculated by the Friedewald equation as follows:

VLDL-C = TGs/5 & LDL-C = TC – HDL-C – VLDL-C [22].

Histological examination

Fixed liver samples embedded in paraffin and cut into 5 µm sections and deparaffinized with xylene. The sections were subjected to standard hematoxylin/eosin (H&E) staining and checked under a light microscope at 40X magnification. Quantitative histological scoring assessed by an expert blind to the study design. Five fields of view of each five sections were evaluated by the histologist (BMZ).

Statistical analysis

Data presented as mean±SEM. One-way ANOVA used to compare the mean differences among groups followed by Tukey's post hoc test. Statistical analysis performed using GraphPad Prism software (Version 7). Group differences with $p < 0.05$ considered statistically significant.

Results

Effects of long-term ice-cold water drink on serum AST, ALT, and ALP levels

No significant differences found in AST and ALT levels among various groups. ALP level however, significantly ($p < 0.05$) increased in CW3M group (616.2 ± 48.37 IU/L) compared to control animals (450.3 ± 34.8 IU/L) (Fig. 1).

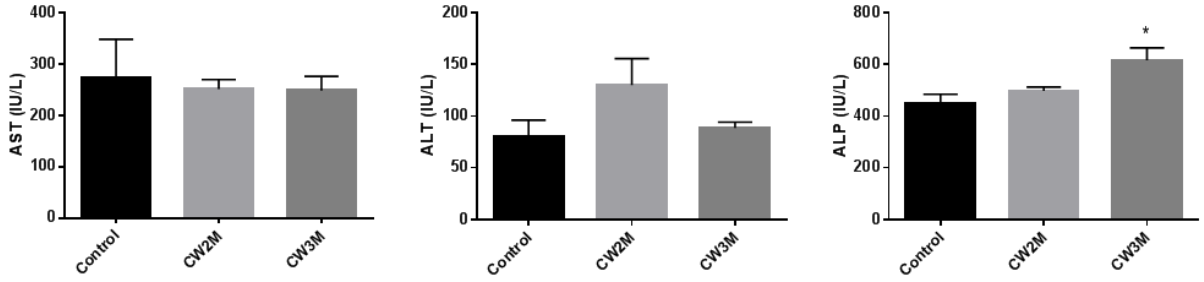

Figure1. Effects of long-term cold-water drink on serum AST, ALT and ALP levels. Data expressed as mean ± SEM, n = 6. * Indicates values significantly different from control group ($P < 0.05$). ALT, Alanine transaminase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase. CW2M, two- month ice-cold water; CW3M, three-month ice-cold water

Effects of long-term ice-cold water drink on fasting blood sugar, serum lipids and lipoproteins

TGs (101.5 ± 8.8 mg/dl) and VLDL-C (20.3 ± 1.7 mg/dl) levels significantly ($p < 0.001$) increased in CW3M group compared to control group (48 ± 8.1 and 9.6 ± 1.6 mg/dl respectively). LDL-C level however, significantly ($p < 0.05$) decreased in CW3M (10.8 ± 3.1 mg/dl) compared to control animals (28.8 ± 4.8 mg/dl).

Fasting blood sugar statistically ($p < 0.05$) lowered in CW3M (123.5 ± 5.9 mg/dl) compared with control animals (184.2 ± 20.4 mg/dl). No significant differences in TC and HDL-C levels found between various groups (Fig. 2).

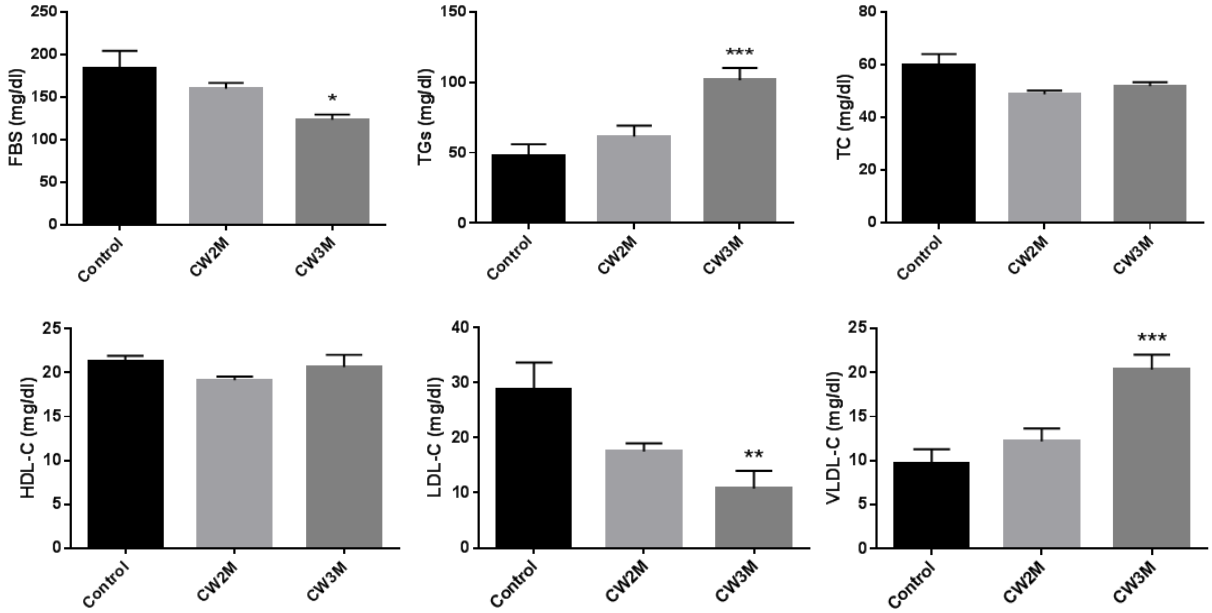

Figure2. Effects of long-term ice-cold water drink on serum FBS, TGs, TC, HDL-C, LDL-C, and VLDL-C levels. Data expressed as mean ± SEM, n = 6. *, ** and *** significantly different from control group ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively). TGs, Triglycerides; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; CW2M, two-month ice-cold water; CW3M, three-month ice-cold water.

Effects of long-term ice-cold water drink on liver histology

Histological studies showed notable (mild to moderate) intracellular accumulation of lipid droplet, proliferation of Kupffer cells and necrosis in cord hepatocytes of liver lobules in CW3M group. Mild changes were also found in CW2M animals (Fig. 3).

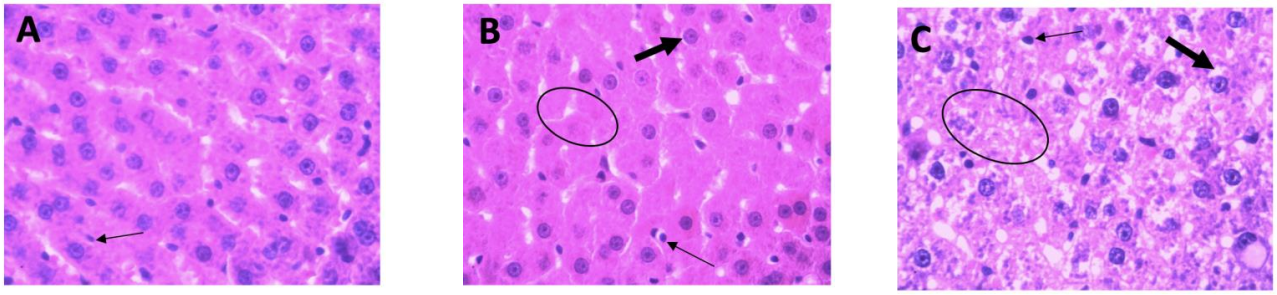

Figure3. Microscopic examination of the liver. (A) Liver in control rats shows normal hepatocyte architecture with no droplet formation, necrosis, cellular cords derangement. Normal Kupffer cell infiltration in sinusoidal wall can also be seen. (B) Liver in CW2M animals shows mild lipid droplet formation, Kupffer cell infiltration, hepatocellular necrosis and cellular cord derangement, (C) liver in CW3M animals show notable (mild to moderate) lipid droplet formation, Kupffer cell infiltration, hepatocellular necrosis and cellular cord derangement. Thick arrows show lipid droplet in the cytoplasm of hepatocyte; thin arrows show Kupffer cells in the sinusoidal wall; and circles show necrotic and cellular cords deranged areas. (H&E staining 40X).

Discussion

Despite daily use of water in mammals, it's hemodynamic, autonomic and metabolic responses have not been studied in detail [8]. The aim of the present work is to study probable role of long-term ice-cold water drink as a dietary lifestyle in NAFLD pathophysiology. NAFLD, like other metabolic-related diseases, has an increasing prevalence [23,24] and is considered as a worldwide health threat [25,26]. To prevent and manage this burden, dietary and medical strategies are worth exploring [27].

The pathogenesis of NAFLD is often interpreted with a 'double-hit' hypothesis. The first hit is lipid accumulation in the hepatocytes which is hallmarked with hepatocellular micro-vesicle formation in our study. The 'second hit' is followed by pro-inflammatory mediators and reactive oxygen species (ROS) formations and resultant inflammatory responses, hepatocellular injury, necrosis and fibrosis [28]. All these changes have been clearly tracked by Kupffer cells infiltration, necrosis and cellular cord derangement reported in the present study.

Proofs are growing for Kupffer cells critical contribution in NAFLD progression [29]. Kupffer cells can both be activated via gut flora modifications, gut barrier malfunction and toxic lipid internalization of the adjacent hepatocytes or Kupffer cells themselves. This activation also plays a role in insulin resistance, fibrosis development and inflammation [30].

Lipid metabolism in the liver is generally altered in NAFLD [29] and regarded as a major risk factor for dyslipidemia [20]. TGs accumulation in hepatocytes is the landmark of NAFLD due to an imbalance between its synthesis and removal. This is also reflected with significant increase in serum TG and VLDL-C levels in this study.

The liver is the source for elevated ALP level in most patients. Increased osteoblastic activity seen in bone disorders is the next likely contributor [31]. NAFLD could be presented with isolated ALP elevation instead of typical elevation of ALP with aminotransferases [32]. Serum aminotransferases may be normal in individuals with NAFLD [33]. Elevated ALP and accompanying liver injury may testify its hepatic source in this study.

Although the precise mechanism of these effects needs further studies, a presumptive one can be activation of the gastrointestinal thermoreceptors [10]. Cold receptor activation of the GI tract inhibits vasopressin release from the paraventricular nucleus of the hypothalamus [15].

The best characterized metabolic actions of vasopressin are related to the liver, an organ rich with its receptors (5 times greater than the kidney). Glycogenolysis is probably the most prominent of the vasopressin hepatic effects, though it also enhances gluconeogenesis and glycolysis, and reduces lipogenesis and ketogenesis [34]. Vasopressin can also inhibit tissue lipase by its direct effect [35]. Conceivably, reduced vasopressin release in long-term ice-cold water drink resulted in tissue lipolysis and hypertriglyceridemia and lipogenesis in the liver with respective mild to moderate steatosis in test groups. Serum glucose can also decrease following reduced vasopressin serum level due

to reduced hepatic glycogenolysis and gluconeogenesis.

It seems that long-term ice-cold water, as stated hundreds of years ago by Avicenna, has deleterious functional and structural effects on the liver. However, we believe the present work is a preliminary study and should be followed by further investigations to clarify the relationship between ice-cold water drink and liver functions and structure changes.

Declaration of competing interest

The authors declare that there was no conflict of interest.

Acknowledgment

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References

[1] Avicenna, Gruner OC, Bakhtiar L, Shah MH. The Canon of Medicine of Avicenna. Kazi Publiations. Chicago 1999; p 164.

[2] Jafari P, Mojahedi M, Zareiyan A, Mokaberinejad R, Chaichi Raghimi M, Hakimi F. Water intake from the points of view of rhazes and avicenna. J Complement Med Res 2019;26:126-132.

[3] Huang WH, Lin YZ, Huang FY. Effect of ice water ingestion on asthmatic children after exercise challenge. Acta Paediatr Tw 2000;41:184-188.

[4] Mattsson P. Headache caused by drinking cold water is common and related to active migraine. Cephalalgia 2001;21:230-235.

[5] Brown CM, Dulloo AG, Montani JP. Water-induced thermogenesis reconsidered: the effects of osmolality and water temperature on energy expenditure after drinking. J Clin Endocrinol Metab 2006;91:3598-3602.

[6] Dubnov-Raz G, Constantini NW, Yariv H, Nice S, Shapira N. Influence of water drinking on resting energy expenditure in overweight children. Int J Obes 2011;35:1295-1300.

[7] Ma J, Hu W, Wu D, Zhang Q, Peng C, Cao K. Drinking cold water increases blood pressure in healthy young students. Blood Press Monit 2014;19:118-119.

[8] Girona M, Grasser EK, Dulloo AG, Montani JP. Cardiovascular and metabolic responses to tap water ingestion in young humans: does the water temperature matter? Acta physiol 2014;211:358-370.

[9] El Ouazzani T, Mei N. Electrophysiologic properties and role of the vagal thermoreceptors of lower esophagus and stomach of cat. Gastroenterology. 1982;83:995-1001.

[10] El Ouazzani T. Thermoreceptors in the digestive tract and their role. J Auton Nerv Syst 1984;10:246-254.

[11] Villanova N, Azpiroz F, Malagelada JR. Gastrogastric reflexes regulating gastric tone and their relationship to perception. Am J Physiol 1997;273:G464-469.

[12] Wang RF, Wang ZF, Ke MY, Fang XC, Sun XH, Zhu LM. Temperature can influence gastric accommodation and sensitivity in functional dyspepsia with epigastric pain syndrome. Digest Dis Sci 2013;58:2550-2555.

[13] Webber DE, Nouri M, Bell FR. A study of the effects of meal temperature on gastric function. Pflügers Archiv 1980;384:65-68.

[14] Golher DM, Thirumurugan P, Patel BH, Upadhyay VK, Sahu S, Gaur GK. Effect of drinking water temperature on physiological variables of crossbred dairy cattle at high altitude temperate region of Himalayas. Vet World 2015;8:1210-1214.

[15] Salata RA, Verbalis JG, Robinson AG. Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. J Clin Endocrinol Metab 1987;65:561-567.

[16] Yamazaki T, Yamori M, Asai K, Nakano-Araki I, Yamaguchi A, Takahashi K. Mastication and risk for diabetes in a Japanese population: a cross-sectional study. PloS One 2013;8:e64113.

[17] Zhu B, Haruyama Y, Muto T, Yamazaki T. Association between eating speed and metabolic syndrome in a three-year population-based cohort study. J Epidemiol 2015;25:332-336.

[18] Papadakis MA, McPhee SJ, Rabow MW. Current medical diagnosis & treatment. McGraw Hill Education. New York 2017.

[19] Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol 2019;70:531-544.

[20] Manne V, Handa P, Kowdley KV. Pathophysiology of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. Clin Liver Dis 2018;22:23-37.

[21] Petta S, Gastaldelli A, Rebelos E, Bugianesi E, Messa P, Miele L. Pathophysiology of Non Alcoholic Fatty Liver Disease. Int J Mol Sci 2016;17:2082.

[22] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[23] Brandt A, Hernandez-Arriaga A, Kehm R, Sanchez V, Jin CJ, Nier A. Metformin attenuates the onset of non-alcoholic fatty liver disease and affects intestinal microbiota and barrier in small intestine. Sci Rep 2019;9:6668.

[24] Likhitsup A, Dundulis J, Ansari S, El-Halawany H, Michelson R, Hutton C. Prevalence of non-alcoholic fatty liver disease on computed tomography in patients with inflammatory bowel disease visiting an emergency department. Ann Gastroenterol 2019;32:283-286.
[25] Afarideh M, Aryan Z, Ghajar A, Ganji M, Ghaemi F, Saadat M. Association of non-alcoholic fatty liver disease with microvascular complications of type 2 diabetes. Prim care Diabetes 2019;13:505-514.
[26] Kempinski R, Lukawska A, Krzyzanowski F, Slosarz D, Poniewierka E. Clinical outcomes of non-alcoholic fatty liver disease: Polish-case control study. Adv Clin Exp Med 2019;28:1615-1620.
[27] Vitaglione P, Mazzone G, Lembo V, D'Argenio G, Rossi A, Guido M. Coffee prevents fatty liver disease induced by a high-fat diet by modulating pathways of the gut-liver axis. J Nutr Sci 2019;8:e15.
[28] Burgio VL, Ballardini G, Artini M, Caratozzolo M, Bianchi FB, Levrero M. Expression of co-stimulatory molecules by Kupffer cells in chronic hepatitis of hepatitis C virus etiology. Hepatology 1998;27:1600-1606.
[29] Baffy G. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. J Hepatol 2009;51:212-223.
[30] Lanthier N. Targeting Kupffer cells in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why and how? World J Hepatol 2015;7:2184-2188.
[31] Sharma U, Pal D, Prasad R. Alkaline phosphatase: an overview. Indian J Clin Biochem. 2014;29:269-278.
[32] Pantsari MW, Harrison SA. Nonalcoholic fatty liver disease presenting with an isolated elevated alkaline phosphatase. J Clin Gastroenterol 2006;40:633-635.
[33] Jameson JL. Harrison's principles of internal medicine. 19th ed. McGraw-Hill Education. New York 2018.
[34] Kovács L, Lichardus B. Metabolic Effects of Vasopressin. Dordrecht: Springer Netherlands 1989.
[35] Koshimizu T, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A. Vasopressin V1a and V1b Receptors: From Molecules to Physiological Systems. Physiol Rev 2012;92:1813-1864.