

COMMENTARY

Association of Repeatedly Heated Oil-Induced Hepatic Fat Accumulation and Development of Cancer: A Commentary

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Abstract: This commentary refers to our recently published article “Association of long-term consumption of repeatedly heated mix vegetable oils (RHMVO) in different doses and hepatic toxicity through fat accumulation.” As highlighted in this article, long-term intake of RHMVO leads to fat accumulation in hepatocytes, oxidative stress, and lipid peroxidation. Several studies have illustrated the negative effect of RHMVO on human health. In this commentary, we considered other recent evidence reporting the carcinogenic and mutagenic potential of RHMVO. Furthermore, we emphasized the unique and easy to perform parameters to measure oxidative stress, lipid peroxidation, fat accumulation, and increased inflammation in hepatocytes to identify progression toward hepatic carcinoma. Our commentary is also intended to further highlight the necessity of developing food policies and regulatory bodies as an approach to minimize the increasing trend of cancer incidence.

Keywords: Edible oils, Repeated heating, Oxidative stress, Thermal oxidation

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1. Background

Our recently published article^[1] has highlighted the strong association between the use of repeatedly heated mix vegetable oils (RHMVO) and the excessive production of reactive oxygen species (ROS) that overwhelm the cellular antioxidant defense capacity resulting in oxidative stress, a proven key factor for cardiovascular diseases, carcinogenesis, and neurodegenerative diseases. As the vital food component, vegetable oils serve as the building block of biomembranes and precursor of several hormones^[2]. To minimize the cost, vegetable oils are reused after food processing in South Asian countries. Fatty acids (FAs) that naturally present in cis-isomers arrangement but could be converted into trans-isomer arrangement during the process of thermal oxidation have physical properties of saturated FAs^[3,4]. Long-term use of trans-isomer FAs leads to metabolic syndrome, a complex condition of overweight, cardiovascular diseases, insulin resistance, and systemic inflammations^[5].

Fatty liver disease or hepatic steatosis (not due to alcohol consumption), which is also known as non-alcoholic fatty liver disease (NAFLD), is generally linked to metabolic syndrome. About 34% of healthy adult Americans and 29% of Japanese presented with mild hepatic asymptomatic steatosis manifested by histological

presentation of NAFLD to severe inflammation will develop hepatocellular injury and fibrosis (termed as non-alcoholic steatohepatitis, abbreviated as NASH) that progresses to other complications, such as cirrhosis with hepatic failure and hepatocellular carcinoma^[6].

Accumulating evidence is emerging that the food composition plays an important role in the development of hepatocellular carcinoma and alterations in our diet could prevent it. At present, dietary recommendations are in place, providing guidelines about the appropriate dietary consumption to reduce hepatic fat accumulation, but the cooking method and time with regard to oils were not addressed^[7]. In addition, lack of appropriate food policies and functioning regulatory bodies is another big challenge in developing countries.

2. Animal model for RHMVO consumption and hepatic toxicity through fat accumulation

In our previous study, we managed to develop an animal model (rabbit) that mimics the human scenario^[1]. Since the oils could be used differently on daily basis, the rabbits were treated with RHMVO and single time heated mixed vegetable oils. In addition, other groups of rabbits were given low and high doses of the oils in the same study to address the fact that oil consumption could vary among different individuals. The third and the most important aspect of the study is that we administered the oils for longer durations (16 weeks) to recapitulate the long-term use of the oils in humans, reflecting the deleterious effects of edible oils if used chronically. A mixture of oils was used to imitate the Pakistani population scenario, where the olive, canola, and sunflower oils blend are most used.

3. Hepatic triglyceride accumulation-induced liver damage

Excessive hepatic fat accumulation or NAFLD is histologically confirmed with the presence of a minimum of 5% of fat accumulated in hepatocytes. Besides, the presence of hepatocellular ballooning with lobular inflammation and typical pericellular or perisinusoidal fibrosis further confirms the diagnosis of NASH^[6]. The exact mechanism of hepatocellular damage associated with the accumulation of hepatic triglycerides is incompletely understood, but insulin resistance is a significant trigger of the increased influx of hepatic free FA and triglyceride production in the liver. Hyperglycemia and hyperinsulinemia also promote lipogenesis by upregulating carbohydrate response element-binding proteins and sterol regulatory element-binding protein-1c. Furthermore, reduced apolipoprotein B synthesis and its defective incorporation with triglycerides may also lead to impaired lipid export from the liver^[6].

4. Excessive hepatic fat accumulation and subsequent lipid peroxidation and oxidative stress

A comparatively practical and cost-effective approach was adopted in the study to evaluate the hepatic toxicity of the thermally oxidized oils. Uninhibited lipid peroxidation induced by oxidative stress may lead to cellular injuries by damaging protein and DNA^[8,9]. Thus, malondialdehyde (MDA), a stable lipid peroxidation end-product, was measured to estimate the overall lipid peroxidation. In addition, superoxide converts to hydrogen peroxide and then quickly is degraded to water by the first-line antioxidant defense mechanism comprising superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Thus, the levels of SOD, CAT, and GPx were studied since they are biological oxidative stress markers^[10,11]. Differences in fat accumulation and inflammatory response were confirmed by histological examination.

We reported that long-term consumption of RHMVO elevated all the important serum liver function markers in rabbits, indicating ROS-induced hepatic injury that further leads to hepatitis, cirrhosis, and hepatic tumor^[4,10]. An important finding in this study is the significant elevations of MDA, a lipid peroxidation marker, in the RHMVO-fed animals. Other studies in addition to our article also support that lipid peroxidation starts in the biological system through the elimination of hydroxyl radical from a -CH₂- group of polyunsaturated FAs. Then, molecular rearrangement produces conjugated dienes that interact with oxygen and generate peroxyl radicals. Lipid hydroperoxide, cyclic peroxide, and MDA are produced when peroxyl radicals interact with the hydroxyl radical moiety of lipids^[10]. As a result of lipid peroxidation, biological membranes are disturbed, initiating the impairment of structure and function. Finally, the production of ROS is promoted due to the altered signaling mechanisms^[12], corroborating the role of augmented oxidative stress consequent to long-term intake of RHMVO as the major contributing factor of carcinogenesis.

5. Carcinogenic potential of long-term use of repeatedly heated oils

The 7th International Symposium on Deep-Fat Frying highlighted the importance of deeper understanding into the deleterious properties of oxidized lipids as well as the toxicity and carcinogenicity of chemicals generated through deep-frying, thereby warranting more research^[13]. Cam *et al.* have reported in their recent study that food processing through deep-frying which leads to chemical alteration of lipids is also a common practice in the United States^[14]. They used a late-stage breast cancer murine model and monitored the metastasis of 4T1 tumor cells using bioluminescent imaging. The animal model was developed; one arm was fed with fresh soybean oil and another thermally oxidized

oil. They also reported that both bioluminescent and histological investigations confirmed the significant growth of metastatic lung tumor cells and increase in cellular proliferation marker (Ki-67) in animals fed with thermally oxidized oil. Furthermore, they also revealed that thermally oxidized oil could modulate hepatic gene expressions. Cam *et al.* developed a preclinical model for studying the late stage of breast cancer. They observed metastasis in the lung, liver, and lymph node in animals fed with a diet containing 10% w/w thermally oxidized vegetable oils, and concluded that long-term use of thermally oxidized vegetable oils can cause different malignancies^[14].

A recently published critical review focusing on the impact of consumption of RHMVO on the incidence of various cancers has reported that repetitive oil heating produces a large amount of polycyclic aromatic hydrocarbons, which have been reported to contain significant mutagenic and carcinogenic potentials depending on the cycles and time duration the oil is repeatedly heated^[15]. This review focused on the tumorigenic, mutagenic, genotoxic, and carcinogenic potential of chronic intake of RHMVO and their fumes. Zhao *et al.* also evaluated that the intake of RHMVO could increase the susceptibility for lung cancer due to the lack of ventilatory system in the home kitchens^[16]. Kohlmeier *et al.* have reported that the trans-FA is a major risk factor of causing breast cancer^[17]. Of particular concern, hydrogenated oils, which contain trans-FAs, are commonly consumed in domestic and commercial food processing.

6. Hepatic fat accumulation and hepatocarcinogenesis

While supporting the evidence of carcinogenic effects of long-term intake of RHMVO in both low and high doses, we emphasized in the same article that the consumption of RHMVO is related to a significant reduction of enzymatic antioxidants in the liver by inducing oxidative stress and pro-inflammatory state, and found that the SOD, GPx, and CAT levels were significantly reduced in the RHMVO-fed animals. Halliwell and Gutteridge reported that modified antioxidant activities lead to DNA and protein oxidation and, finally, cellular death^[10]. High-fat diet consumption produces toxic intermediates that inhibit antioxidant enzyme activity^[18]. Accumulation of hydrogen peroxide and ROS also promotes the rapid synthesis of hydroxyl radicals. These findings recommend that long-term intake of thermally oxidized vegetable oils is capable of inducing significant genotoxic and carcinogenic effects^[19,20]. With time, these ongoing inflammatory changes and lipid peroxidation cause malignant liver disease. In another publication, we have reported that long-term use of RHMVO significantly raised the oxidative stress and lipid peroxidation markers in rabbits, and the liver weight of the rabbits fed on RHMVO was significantly higher than that of the control after 16 weeks^[21]. We further confirmed the significant fat accumulation in the

liver through histopathological examination of hepatocytes^[1].

In addition to oxidative stress, stellate cell activation, microvascular, and mitochondrial injuries are among other factors that contribute to the pathogenesis of hepatic fibrosis or cirrhosis. Nevertheless, the incidence of hepatocellular carcinoma rises with the progression of cirrhosis^[4,10]. A high incidence of hepatocellular carcinoma in overweight individuals with cirrhosis has been reported^[22]. The previous studies have supported hepatocarcinogenesis in cirrhosis patients experiencing recurring hepatocyte death and compensatory regeneration mechanism through continuous cellular regrowth and proliferation that ultimately favors in developing tumors^[23,24]. Hepatic steatosis and insulin resistance also favor hepatocarcinogenesis through promoting adipose tissue-derived inflammatory reactions, oxidative stress, lipotoxicity, hormonal changes, and stimulation of the insulin-like growth factor-1 axis by hyperinsulinemia. Other possible, important, and clinically pertinent aspects include diet, intestinal microbiomes, and genetic factors^[24].

A constant phenomenon of chronic inflammation due to excessive hepatic fat accumulation and insulin resistance encourages macrophage recruitment and excessive release of numerous pro-inflammatory cytokines, including tumor necrosis factor- α (TNF α) and interleukin-6 (IL-6) which are significantly related to the progression toward hepatocarcinogenesis, mainly through their action on the inhibitors of nuclear factor kappa-B kinase subunit beta (IKK) and Jun N-terminal kinase (JNK) signaling pathway. These pro-inflammatory cytokines are the key mediators of fat accumulation and obesity-induced inflammatory cascades and, therefore, play an important role in hepatocarcinogenesis. TNF α -induced activation of JNK leads to impairment of the regular signaling mechanism of insulin receptors^[25], and the activated JNK further interacts with NF- κ B to promote the transcription of genes that are mainly engaged in apoptosis, inflammation, proliferation, and angiogenesis. IL-6-induced activation of signal transducer and activator of transcription 3 (STAT3) promotes cellular differentiation and growth^[26]. The higher IL-6 levels in individuals with fat accumulation and STAT3 involvement in several tumors indirectly highlight the importance and role of these pathways in hepatocarcinogenesis^[24-26].

7. Conclusion and further directions

It is known that the sensitivity and specificity of diagnostic techniques of different fatty liver disease are mostly not 100%, especially in the obese patients, and even when the more sensitive diagnostic approaches are used, successful diagnoses can only be made when the fatty liver diseases have reached the end-stage^[6]. Therefore, the clinical implication of our work is significant for NAFLD diagnosis that employs oxidative stress markers in the earlier stages^[1].

We also conclude that the carcinogenic effects of

long-term use of thermally oxidized oils need to be made known not only in the developing countries but also in the developed ones. Through this commentary, we further emphasize the need to study the association of the long-term consumption of thermally oxidized oils with cancers of the liver and other organs. The new insights may spawn additional approaches to reduce the risk of occurrence and recurrence of cancer through diet modifications. Developing food policies and strengthening regulatory bodies may also help to achieve this purpose.

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Conflicts of interest

The authors of this article do not have any commercial or financial conflicts of interest.

Author contributions

G.A. and Z.N. wrote the initial draft. A.S. and K.H. finalized the manuscript after critical revisions. All authors approved the final manuscript before submission.

References

1. Ambreen G, Siddiq A, Hussain K, 2020, Association of Long-term Consumption of Repeatedly Heated Mix Vegetable Oils in Different doses and Hepatic Toxicity through fat Accumulation. *Lipids Health Dis*, 1–9.
2. Vašková H, Bučková M, 2015, Thermal Degradation of Vegetable Oils: Spectroscopic Measurement and Analysis. *Proc Eng*, 100:630–5.
3. Sommerfeld M, 1983, Trans Unsaturated Fatty Acids in Natural Products and Processed Foods. *Prog Lipid Res*, 22:221.
4. Niu SL, Mitchell DC, Litman BJ, 2005, Trans Fatty Acid Derived Phospholipids Show Increased Membrane Cholesterol and Reduced Receptor Activation as Compared to their Cis Analogs. *Biochemistry*, 44:4458–65.
5. Sun Q, Ma J, Campos H, et al., 2007, A Prospective Study of Trans Fatty Acids in Erythrocytes and Risk of Coronary Heart Disease. *Circulation*, 115:1858–65.
6. Adams L, Angulo P, 2005, Recent Concepts in Non-alcoholic Fatty Liver Disease. *Diabetic Med*, 22:1129–33.
7. Koumbi L, 2017, Dietary Factors can Protect against Liver Cancer Development. *World J Hepatol*, 9:119.
8. Nakai A, Oya A, Kobe H, et al., 2000, Changes in Maternal Lipid Peroxidation Levels and Antioxidant Enzymatic Activities before and after Delivery. *J Nippon Med Sch*, 67:434–9.
9. Spiteller G, 2007, The Important Role of Lipid Peroxidation Processes in Aging and Age Dependent Diseases. *Mol Biotechnol*, 37:5–12.
10. Halliwell B, Gutteridge JM, 2015, Free Radicals in Biology and Medicine. Oxford, USA: University Press.
11. Ighodaro O, Akinloye O, 2018, First Line Defence Antioxidants-superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX): Their Fundamental Role in the Entire Antioxidant Defence Grid. *Alexandria J Med*, 54:287–93.
12. Brown DA, 2006, Lipid Rafts, Detergent-resistant Membranes, and Raft Targeting Signals. *Physiology*, 21:430–9.
13. Gertz C, Stier RF, 2013, 7th International Symposium on Deep-Fat Frying, San Francisco, CA (USA): Recommendations to Enhance Frying. *Eur J Lipid Sci Technol*, 115:589–90.
14. Cam A, Oyirifi AB, Liu Y, et al., 2019, Thermally Abused Frying Oil Potentiates Metastasis to Lung in a Murine Model of Late-stage Breast Cancer. *Cancer Prevent Res*, 12:201–10.
15. Ganesan K, Sukalingam K, Xu B, 2019, Impact of Consumption of Repeatedly Heated Cooking Oils on the Incidence of Various Cancers-a Critical Review. *Crit Rev Food Sci Nutr*, 59:488–505.
16. Zhao Y, Wang S, Aunan K, et al., 2006, Air Pollution and Lung Cancer Risks in China a Meta-Analysis. *Sci Total Environ*, 366:500–13.
17. Kohlmeier L, Simonsen N, vant Veer P, et al., 1997, Adipose Tissue Trans Fatty Acids and Breast Cancer in the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. *Cancer Epidemiol Biomarkers Prev*, 6:705–10.
18. Thampi B, Manoj G, Leelamma S, et al., 1991, Dietary Fiber and Lipid Peroxidation: Effect of Dietary Fiber on Levels of Lipids and Lipid Peroxides in High Fat Diet. *Indian J Exp Biol*, 29:563–7.
19. Srivastava S, Singh M, George J, et al., 2010, Genotoxic and Carcinogenic Risks Associated with the Dietary Consumption of Repeatedly Heated Coconut Oil. *Br J Nutr*, 104:1343–52.
20. Srivastava S, Singh M, George J, et al., 2010, Genotoxic and Carcinogenic Risks Associated with the Consumption of Repeatedly Boiled Sunflower Oil. *J Agric Food Chem*, 58:11179–86.
21. Siddiq A, Ambreen G, Hussain K, et al., 2019, Oxidative Stress and Lipid Per-oxidation with Repeatedly Heated Mix Vegetable Oils in Different doses in Comparison with Single Time Heated Vegetable Oils. *Pak J Pharm Sci*, 32:2099–105.
22. Farrell GC, Larter CZ, 2006, Nonalcoholic Fatty Liver Disease: From Steatosis to Cirrhosis. *Hepatology*, 43:S99–112.
23. Liu Q, Bengmark S, Qu S, 2010, The Role of Hepatic fat Accumulation in Pathogenesis of Non-alcoholic Fatty Liver Disease (NAFLD). *Lipids Health Dis*, 9:1–9.
24. Marengo A, Rosso C, Bugianesi E, 2016, Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Ann Rev Med*, 67:103–17.
25. Hirosumi J, Tuncman G, Chang L, et al., 2002, A Central Role for JNK in Obesity and Insulin Resistance. *Nature*,

- 420:333–6.
26. Hodge DR, Hurt EM, Farrar WL, 2005, The Role of IL-6 and STAT3 in Inflammation and Cancer. *Eur J Cancer*, 41:2502–12.