Red and Processed Meat Intake in Relation to Non-Alcoholic Fatty Liver Disease Risk: Results from a Case-Control Study

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ABSTRACT

Data on the association between dietary red meat intake and non-alcoholic fatty liver disease (NAFLD) are limited. We designed this case-control study to determine the association between red and processed meat consumption and risk of NAFLD in Iranian adults. A total of 999 eligible subjects, including 196 NAFLD patients and 803 non-NAFLD controls were recruited from hepatology clinics in Tehran, Iran. A reliable and validated food frequency questionnaire was used to evaluate the red and processed meat intakes. The analyzes performed showed that in an age- and gender-adjusted model, patients with the highest quartile of red meat intake had an approximately three-fold higher risk of NAFLD than those with the lowest quartile of intake (odds ratio [OR], 3.42; 95% confidence interval [CI], 2.16–5.43; p value < 0.001). Moreover, patients in the highest quartile of processed meat intake had a 3.28 times higher risk of NAFLD, compared to the lowest quartile(OR, 3.28; 95% CI, 1.97–5.46; p value < 0.001). Both these associations remained significant by implementing additional adjustments for body mass index, energy intake, dietary factors, diabetes, smoking, and physical activity (OR, 3.65; 95% CI, 1.85–7.18; p value < 0.001 and OR, 3.25; 95% CI, 1.57–6.73; p value = 0.002, respectively).Our findings indicate that both red and processed meat intakes are related to the increased odds of NAFLD; however, prospective studies are needed to confirm these results.

Keywords: NAFLD; Fatty liver; Red meat; Meat products; Case Control studies

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered as a prevalent health concern among subjects with no significant alcohol intake. It manifests itself in the accumulation of fat in the liver, which can progress from simple steatosis to steatohepatitis, eventually cirrhosis [1,2]. Because of changes in lifestyle, the number of obese or diabetic patients at risk of NAFLD is growing rapidly [3,4]. Estimates in Iran show that about 33.9% of Iranian adults have NAFLD [5]. Diabetes, hyperlipidemia, central obesity, sedentary lifestyle are known as main NAFLD risk factors [6-8].
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Conflict of Interest
The authors declare that they have no competing interests.

Author Contributions
Conceptualization: Hekmatdoost A, Rahimi-Sakak F; Data curation: Hekmatdoost A; Formal analysis: Hekmatdoost A, Emamat H; Funding acquisition: Rahimi-Sakak F; Investigation: Rahimi-Sakak F, Maroofi M; Methodology: Hekmatdoost A, Emamat H; Supervision: Hekmatdoost A; Writing - original draft: Rahimi-Sakak F, Maroofi M; Writing - review & editing: Emamat H, Hekmatdoost A.

Meat and NAFLD

So far, some dietary factors have been demonstrated to be associated with NAFLD risk [9-14]. Recent reports suggest that higher meat intake is associated with an increased risk of obesity [15,16], diabetes mellitus [17], cancer and other chronic diseases [18]. On the other hand, red and processed meats are rich in saturated fatty acids (SFA), cholesterol, and sodium [18]. Animal studies suggested that diet rich in trans fatty acids (TFA) and cholesterol may induce NAFLD [19]. In addition, evidences revealed that NAFLD patients consume less dietary fiber [20], fish, mono unsaturated fatty acid (MUFA) in comparison to healthy people [21]. However, the association of dietary red meat and processed meat with NAFLD risk has not been studied extensively. The cross-sectional studies have shown a positive association between meat intake and the odds of NAFLD [22,23]. Also recently, Hashemian et al. [24] found in the Golestan Cohort Study that subjects who ate more red meat were more likely to develop NAFLD. Therefore, we hypothesized that there may be a link between the risk of NAFLD and dietary intake of red and processed meats. Therefore, we designed this case-control study to evaluate this possible association.

MATERIALS AND METHODS

Study design and patients
The full details of the current survey have been previously described [11,25]. The study protocol was approved by Shahid Beheshti University of Medical Sciences ethics committee (ethical code: IR.SBMU.REC.1397.101). A total of 196 recently identified NAFLD patients and 803 healthy subjects have participated in our case-control study. A gastroenterologist was responsible for the diagnosis of NAFLD in patients. Identification of NAFLD was based on the presence of hepatic steatosis via ultrasound and Fibroscan procedures. Controls did not have any evidence of hepatic steatosis. The inclusion criteria for cases were diagnosis of NAFLD by a gastroenterologist, CAP score > 263 in Fibroscan exam, age older than 18 years and consumption of alcohol less than 20 g/day in women and less than 30 g/day in men. The inclusion criteria for controls were older than 18 years and no evidence of steatosis in hepatic ultrasound examination. Participants who were on a special diet or completed less than 90% of the food frequency questionnaire (FFQ), or subjects with a daily energy intake greater than or equal to 4,500 kcal/day or less than 500 kcal/day were excluded. Convenience sampling was used as a way of selecting participants. The rate of participation in our study for cases and controls were about 94% and 98%, respectively. Groups were matched by age range of 5 years and all participants were enrolled in the study at the same clinic. Written informed consent was obtained from all subjects at the beginning of the study.

Assessment of dietary data
Dietary intake was assessed based on the FFQ by a trained healthcare professional. A validated and reliable FFQ questionnaire was used and included 168 commonly consumed foods in Iran with standard serving sizes [26]. We used daily, weekly, or monthly criteria to rate the frequency of consumption of each food item. Then, we converted all of them to daily consumption. In the case of red meat (beef or lamb or ground red meat) and processed meat (sausages or burgers), we classified the participants to quartiles according to the amount of red and processed meat intakes. NUTRITIONIST V was used to assess the dietary nutrients intakes.

Assessment of other data
The metabolic equivalent task was used to assess the individual’s physical activity [27]. Age, sex, drug history, medical status, smoking behavior, and alcohol consumption were evaluated...
using the questionnaires. Participant’s weight and height were measured without shoes or any heavy clothes. Body mass index (BMI) was calculated.

**Statistical analysis**

The Statistical Package for Social Sciences (version 20.0; SPSS, Chicago, IL, USA) was used for the analysis. We assessed the normality of the variables using histogram charts and Kolmogorov–Smirnov analysis, indicating that all of the variables had a normal distribution. The $\chi^2$ test was used to check differences in the distribution of categorical variables, and independent t-test was used to assess differences in the distribution of continuous variables. Participants were categorized according to quartiles of red and processed meat intakes. First quartile was considered as the reference category for all logistic regression analyses. To estimate the risk of NAFLD across quartiles of red and processed meat intakes, multivariable logistic regression models were used with NAFLD as the dependent variable and red or processed meat as an independent variable; the odds ratios (ORs) and 95% confidence intervals (CIs) were reported. Logistic regression models were adjusted for gender, age, BMI, daily intakes of energy, dietary factors (including vegetables, fruits, dairy, nuts and legumes, cereals, soft drinks and sweets, total dietary fiber, MUFA/SFA and sodium), diabetes, smoking, and physical activity. p values < 0.05 were considered to be statistically significant.

**RESULTS**

**Table 1** shows baseline characteristics of case and control groups. In our study, the distribution of age and sex was similar; the mean age of the participants was 43.54 ± 14.13 years and 41.5% (395) of subjects were male. As shown in **Table 1**, diabetes rates were higher among those with NAFLD (p < 0.001). These patients had also significantly higher BMI and less physical activity than controls (p < 0.001).

**Table 2** shows the dietary intakes of participants with NAFLD and control group. Patients with NAFLD consumed more red meats, processed meats, vegetables, fruits, fish, dairy, low-fat dairy, soft drinks and sweets, protein, total dietary fiber, MUFA/SFA and sodium, but lower nuts and legumes, cereals and fats (p < 0.005). There were not any significant differences in other dietary intakes between two groups.

**Table 3** shows the association between red meat consumption and the risk of NAFLD. In age and gender-adjusted model, the risk of NAFLD in quartile 4 was about three times higher than quartile 1 (OR, 3.42; 95% CI, 2.16–5.43; p value < 0.001). After adjusting for BMI, energy intake, dietary factors, diabetes, smoking, and physical activity, this association was stronger and significant (OR, 3.65; 95% CI, 1.85–7.18; p value < 0.001).

| Table 1. Baseline characteristics of non-alcoholic fatty liver disease patients and control subjects |
|---------------------------------------------------------------|
| **Variables** | **Case (n = 196)** | **Control (n = 803)** | **p value** |
| Age (yr)       | 42.3 ± 11.9   | 43.5 ± 14.5   | 0.214 |
| Male (%)       | 51.5          | 41            | 0.007 |
| Body mass index (kg/m$^2$) | 35.7 ± 10.6 | 27.7 ± 4.5 | < 0.001 |
| Physical activity (MET [hr/day]) | 31.0 ± 3.2 | 34.2 ± 3.1 | < 0.001 |
| Diabetes (%)   | 16.6          | 6.8           | < 0.001 |

Data are presented as mean ± SD for continuous variables and percent for categorically distributed variables. MET, metabolic equivalent task.
The association between processed meats intake and the risk of NAFLD is shown in Table 4. In the first model, after controlling for age and sex, there was an increased risk of NAFLD in the third quartile, and patients in the highest quartile had a 3.28-fold higher risk of NAFLD compared to the other quartiles (OR, 3.28; 95% CI, 1.97–5.46; p value < 0.001). In the final model, a significant association was also found in both third and fourth quartiles (OR, 2.36; 95% CI, 1.19–4.65; p value < 0.05 and OR, 3.25; 95% CI, 1.57–6.73; p value = 0.002).

**DISCUSSION**

The results of the current study have shown that higher red or processed meat consumption can notably increase the risk of NAFLD. To our knowledge, there are only three observational...
studies, which had reported similar results. Peng et al. [23], in a cross-sectional study of Chinese participants over the age of 45, examined the association between meat consumption and odds of NAFLD and they found a positive association. Also, in this study, red meat consumption was correlated with serum levels of liver enzymes. Another cross-sectional study looked at meat preparation methods and intake of heterocyclic amines (HCA), and found that unhealthy cooking methods and intake of HCA were positively associated with insulin resistance, a major underlying cause of NAFLD [22]. Recently, a large cohort study of 50,045 Iranian participants in the age range of 40 to 75 years had similar results to our results [24]. Average intake of red/processed meats were higher in studies that performed in non-Iranian population [22,23]. It is recommended to limit the consumption of red meat to less than 100 g/day for cardio-metabolic health [28], and the average consumption of red meat in our study and another study conducted in Iran [24] is less than the recommended values. This finding shows that even in communities with a low average red meat intake, people in the upper consumption categories are at risk for NAFLD. There are some other related studies, which are also in line with our results. A cross-sectional study on Brazilian subjects showed that intake of meats, fats, sugars, beans, and vegetables in NAFLD patients are higher than the recommended amounts [29]. Moreover, it has been shown that a diet rich in red meats and TFA or a western diet, which is full of animal-derived products can simply worsen the progression of NAFLD [21,30].

Our study showed that the association between dietary red/processed meats with NAFLD risk is independent of other known risk factors because the association remained significant after adjusting for BMI, energy intake, dietary factors, diabetes, smoking, and physical activity. Interestingly, the association between red meats consumption and NAFLD became stronger when we adjusted for known confounders. Another interesting finding of this study was that NAFLD risk increased significantly when the consumption of red and processed meats was more than 43.7, and 6.58 g/day, respectively. There was no significant association with low red meat/processed meat intake. This finding suggests that a threshold exists for the risk of red meat/processed meat for NAFLD, whereas the risk is low when consumed in small amounts. Zelber-Sagi et al. [22] have suggested that the association between red/processed meats and risk of NAFLD is due to production of HCAs during cooking of them. They found a positive relationship between HCAs and insulin resistance, but not NAFLD risk. An association between NAFLD and meat consumption has been suggested due to the high heme iron content of meat [17], which causes cellular oxidative stress which decreases insulin action. A meta-analysis has reported that heme-iron intake was associated with 30% higher risk of diabetes [31]. Another mechanism for this association could be high amount of nitrates in processed meats [32]. Nitrites and nitrates used for the preservation of processed meat can be converted into nitrosamines, which promote insulin resistance and diabetes [33]. Furthermore, it has been shown that higher SFA intake may increase liver fat accumulation, which may be due to increased TG levels through suppressing the TWEAK gene, a member of the TNF superfamily expression leading to liver injury [34]. On the other hand, higher sodium content of processed meats may aggravate the risk of NAFLD via obesity and fatness mediation. Also, previous evidence showed that higher sodium intake may exacerbate abnormal metabolic profiles, which are potential elements in NAFLD development [35,36].

This study has some limitations. First, we have done a case-control study, which is not able to define a cause-effect association of red and processed meat intake with NAFLD. Second, FFQ was used as a measurement tool to examine the dietary intakes of individuals, which has some unpreventable errors such as recall bias and measurement bias. However, this study is
of great significance in the sense that it has a large sample size, was conducted in patients with recently diagnosed NAFLD who had not yet received diet or other treatment, and the study was applicable to populations in developing countries with a wide range of dietary intakes and special diets.

CONCLUSION

Our data demonstrate that both red and processed meat intakes are related to the risk of NAFLD. This study was performed on a middle-aged Iranian population and the results may not be generalized to other populations. Therefore, further investigations are required to confirm these findings in other populations, while elucidating the potential mechanisms.

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