Journal of Environmental Health and Sustainable Development

# The Association between Dietary Nitrate Intake and Alanine Transaminase in Adolescent Girls

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***ARTICLE INFO***

***ORIGINAL ARTICLE***

***Article History:***
*Received: 05 June 2022*
*Accepted: 10 August 2022*

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***Keywords:***
*Nitrates,*
*Alanine Aminotransferase,*
*Aspartate Aminotransferases,*
*Alkaline Phosphatase,*
*Gamma-Glutamyl Transferase.*

***ABSTRACT***

***Introduction:*** The effects of dietary nitrate on health are controversial. The current study aims to investigate the relationship between dietary intake of nitrate and liver enzymes among Iranian adolescent girls.

***Materials and Methods:*** This cross sectional study was conducted on 733 adolescent girls. They were recruited from several schools in different areas in the cities of Mashhad and Sabzevar, northeast region of Iran, by random cluster sampling method. The dietary intake of nitrate was assessed using a validated food-frequency questionnaire (FFQ). Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were measured by auto-analyzer. Linear regression was applied to investigate the correlation between nitrate intake and liver enzymes in crude and adjusted models.

***Results:*** There was a direct association between dietary intake of nitrate and serum levels of ALT in crude [$\beta = 0.117$; 95% CI (0.003-0.016); $P < 0.01$] and adjusted models for energy intake, age, BMI percentile, physical activity, menstruation, father's education, and mother's education [$\beta = 0.128$; 95% CI (0.003-0.016); $P < 0.01$]. No significant associations were found between dietary intake of nitrate and levels of ALP, AST, and GGT in crude or adjusted models.

***Conclusion:*** There was a direct relationship between dietary intake of nitrate and serum concentration of ALT. Longitudinal studies are required to examine the association between dietary nitrate intake and liver functional tests.

**Citation:** Darabi Z, Gordon A Ferns, Ghayour-Mobarhan M, et al. ***The Association between Dietary Nitrate Intake and Alanine Transaminase in Adolescent Girls.*** J Environ Health Sustain Dev. 2022; 7(3): 1767-72.

## Introduction

Inorganic nitrate has conventionally been viewed as an inert contaminant in food and water [1.] However adverse effects of nitrate on human health are arguable[2]. Vegetables, especially green, leafy, and root vegetables and processed meats are the main source of dietary nitrate [3, 4]. Results of a cohort study have reported that dietary intake of nitrate from vegetables can reduce the risk of ischemic heart disease, heart failure, peripheral artery disease [5]. However, findings from Nurses' Health Study have shown that dietary nitrate intake was not associated with risk of coronary heart disease (CHD) [6.] Human interventional and experimental studies have reported useful effects of nitrate, such as reducing blood pressure, improvement of endothelial function, glucose tolerance, and lipid profile [7-10.] It should be considered that intake of nitrate or nitrite may also be related to the formation of methemoglobin

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[ DOI: 10.18502/jehsd.v7i3.10727 ]

in blood. This component has had harmful health effects especially on infants [11]. Findings from previous studies about the association between nitrate intake and liver function are inconsistent [12, 13]. Some animal studies have shown that high exposure to nitrate leads to histological, functional, and pathological changes, such as hepatic fibrosis and cellular atrophy in liver [12, 13]. On the other hand, nitrate intake can down regulate the regulatory pathways of lipogenesis and fatty acid oxidation, resulting in protective effect on liver [14]. High levels of liver enzymes, such as ALT, GGT, and AST, even within the normal range, have been associated with high risk of type 2 diabetes as well as increased cardiometabolic risk factors [15-17]. Furthermore, the increase in serum liver enzyme levels within the reference range in childhood is related to an adverse cardiovascular risk profile in adulthood [18].

The present study aims to evaluate the association between dietary nitrate intake and level of liver enzymes in adolescent girls.

## Materials and Methods

### *Study population*

This study was cross-sectionally performed on 733 student girls aged 12-18 years in January 2015. Random cluster sampling method was applied to select participants from several schools in different areas in the cities of Mashhad and Sabzevar. The individuals aged between 12 and 18 years, without taking anti-inflammatory, antidiabetic, anti-depressant, or anti-obesity drugs, and not consuming calcium or vitamin D supplement within the last 6 months. A history of autoimmune diseases, hepatic or renal failure, anorexia nervosa or bulimia, cardiovascular disorders, malabsorption, thyroid, cancer, metabolic bone disease, parathyroid, adrenal disease was considered as exclusion criteria. Written informed consent was obtained from all the participants and their parents. The Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, approved this study.

### *Dietary assessment*

A validated food-frequency questionnaire (FFQ) with 147 food items was used to assess dietary intakes. The validity and reliability of the FFQ were approved in previous studies [19, 20]. Face-to-face interview was conducted by a trained dietitian for completing the FFQ. The recorded portion size in the FFQ were converted to grams using household measures and then were entered to the Nutritionist IV software (First Databank Inc., Hearst Corp., San Bruno, CA, USA) for assessing energy and nutrient intakes [21]. Nitrate intake was calculated by multiplying the reported quantity of consumption for each food item (g/day) by its assigned mean nitrate value (mg/g).

### *Biochemical assessment*

Blood samples were taken early in morning between 8 and 10 am while the participants were in the fasted state (14 h overnight fasting). Blood samples were centrifuged (Hettich model D-78532) for 10 min and serum and plasma were separated into two aliquots. Serum samples were stored at −80° C. ALT, AST, GGT, and ALP from serum samples were measured by commercial kits (Pars Azmun, Karaj, Iran) using the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy).

### *Covariate assessment*

Demographic data was collected by experienced interviewers. Also, anthropometric measurements including height, weight, waist circumferences (WC), and waist-to-hip ratio (WHR) were gathered according to standard protocol. All measurements were performed twice and their mean was reported. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Physical activity data were collected by an experienced interviewer. Adolescents' physical activity was assessed by a validated modifiable activity questionnaire (MAQ) [22]. Physical activity level was measured based on metabolic equivalent task minutes per week (1 MET = 3.5 mL $kg^{-1}$ $min^{-1}$ of $O_2$ consumption).

### *Statistical analysis*

The participants were classified into three

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groups across tertiles of dietary intake of nitrate. General characteristics of the participants across tertiles of nitrate intake were presented as means ± SDs for continuous variables, and as numbers and percentages for categorical variables. To explore the differences between tertiles, one-way-ANOVA and Chi square test were used for continuous and categorical variables, respectively. Multivariate linear regression was used to examine the association between nitrate intake and levels of ALT, AST, ALP, and GGT. Age and energy intake adjustments were performed in Model I. BMI percentile was additionally adjusted in Model II. Final adjustments were done for physical activity and menstruation in model III. All statistical analyses were conducted using the SPSS version 23. P-values < 0.05 were considered significant.

## Results

General characteristics of the study population across tertiles of nitrate intake are shown in Table 1. There were no significant differences for age, BMI percentile, WC, WHR, physical activity, menstruation, AST, GGT, and ALP across tertiles of nitrate intake. However, compared to the subjects in the third tertile, subjects in the first tertile of nitrate intake had significantly lower ALT and energy intake.

**Table 1:** General characteristics of the participants by tertiles of nitrate intake

| Variables | T1 (N = 244) | T2 (N = 245) | T3 (N = 244) | P-value* |
|---|---|---|---|---|
| Age(year) | 14.50 ± 1.52 | 14.43 ± 1.54 | 14.60 ± 1.53 | 0.453 |
| BMI Percentile | 46.55 ± 28.97 | 48.79 ± 28.66 | 48.15 ± 29.18 | 0.227 |
| Waist circumference(cm) | 70.03 ± 8.47 | 70.69 ± 9.54 | 70.79 ± 9.25 | 0.608 |
| WHR | 0.76 ± 0.05 | 0.77 ± 0.06 | 0.7656 ± 0.05 | 0.409 |
| Metabolic equivalent for task(h/week) | 45.04 ± 2.99 | 45.30 ± 3.62 | 45.78 ± 3.65 | 0.054 |
| ALT(IU/L) | 10.61 ± 4.99 | 12.03 ± 6.15 | 12.26 ± 8.48 | 0.022 |
| AST(IU/L) | 19.36 ± 5.85 | 20.39 ± 6.32 | 19.74 ± 6.34 | 0.213 |
| GGT(IU/L) | 12.75 ± 9.24 | 12.37 ± 7.38 | 12.67 ± 8.53 | 0.929 |
| ALP(IU/L) | 361.73 ± 196.80 | 364.87 ± 211.87 | 360.82 ± 210.41 | 0.977 |
| Energy intake | 2142.85 ± 670.63 | 2755.21 ± 746.33 | 3239.50 ± 681.82 | < 0.001 |
| Menstruation % (n) | 91.3(221) | 90.1 (219) | 91 (222) | 0.895 |

BMI: Body mass index; WHR: Waist-to-hip ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyltransferase; ALP: Alkaline phosphatase.
Values are means ± SDs
*Obtained from one way Anova for continuous variables and Chi-squared test for categorical variables

The correlation between nitrate intake and levels of liver enzymes are presented in Table 2. There was a positive association between nitrate intake and serum concentration of ALT in crude model [β = 0.117; 95% CI (0.003-0.016); P < 0.01]. Additionally, this positive correlation remained significant after adjustment for confounding factors, including of age, energy intake, BMI percentile, physical activity, menstruation, father's education, and mother's education [β = 0.128; 95% CI (0.003-0.016); P < 0.01]. No statistically significant association was found between dietary intake of nitrate and levels of AST, GGT, and ALP in crude or adjusted model.

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**Table 2:** Linear regression analysis of the correlations between nitrate intake and liver enzymes

| | B (95%CI) | P-value |
|---|---|---|
| **ALT(IU/L)** | | |
| Crude | 0.117 (0.003-0.016) | 0.003 |
| Model I | 0.116 (0.002-0.017) | 0.012 |
| Model II | 0.108 (0.002-0.016) | 0.016 |
| Model III | 0.128 (0.003-0.016) | < 0.01 |
| **AST(IU/L)** | | |
| Crude | -0.016 (-0.007-0.005) | 0.678 |
| Model I | -0.025 (-0.008-0.005) | 0.577 |
| Model II | -0.022 (-0.008-0.005) | 0.613 |
| Model III | -0.016 (-1.169-0.734) | 0.653 |
| **GGT(IU/L)** | | |
| Crude | 0.037 (-0.006-0.013) | 0.456 |
| Model I | 0.070 (-0.004-0.018) | 0.236 |
| Model II | 0.066 (-0.005-0.017) | 0.259 |
| Model III | 0.079 (-0.005-0.018) | 0.187 |
| **ALP(IU/L)** | | |
| Crude | -0.016 (-0.023-0.154) | 0.684 |
| Model I | -0.009 (-0.237-0.191) | 0.834 |
| Model II | -0.006 (-0.230-0.198) | 0.882 |
| Model III | 0.001 (-0.208-0.214) | 0.979 |

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyltransferase; ALP: Alkaline phosphatase.
Model I: Adjusted for energy intake, age.
Model II: Additionally adjusted for BMI percentile.
Model III: Additionally adjusted for physical activity, menstruation, father's education, and mother's education.

## Discussion

The study results showed that dietary intake of nitrate was positively associated with serum ALT, but not with the other liver enzymes. There is no human study examining the association between nitrate intake and liver enzyme. Results of animal studies are controversy regarding the relationship between nitrate intake and liver function. An experimental study has reported that $NO_3^-$ at a dose of 238 mg/kg body weight increased levels of ALP, GGT, and ALT [23]. Ogur et al. reported that rats treated with drinking water containing 400 mg/L nitrate had higher AST and ALT compared to rat with intake of water with 200 mg/L nitrate [13].

Nitrates and nitrites are the precursors of nitric oxide, producing free radical ONOO. [24] Nitro compounds, such as peroxynitrite are composed of nitrate and ONOO, categorized as free radicals, causing liver injury, mitochondrial dysfunction, hepatic inflammation, and liver cell death [25, 26]. Results of a study showed that NO• induces hepatocyte necrosis when cells are expose to redox stress. In this condition, NO• can increase the activation of p53 and DNA damage[27].

Whilst necrotic liver cells can release enzyme, such as ALT, AST, and ALP into the circulation and increase levels of serum enzymes [28], there was no increase in levels of liver enzymes other than ALT.

Some reports have indicated that nitrate downregulated lipogenesis gene expression, such as sterol regulatory element-binding protein 1 (SREBP1c), acetyl-CoA carboxylase (ACC), and peroxisome proliferator-activated receptor γ coactivator 1 (PGC1α), and had a preventive effect on liver steatosis [14, 29].

This study has several strengths. To the best of the authors' knowledge, it is the first study that investigated the relationship between nitrate intake and levels of liver enzymes in humans. Secondly, this study was conducted on a large sample. Thirdly, the association was adjusted for a wide range of potential confounders. However, the current study has some limitations. This study had

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a cross-sectional design and the causal link between nitrate intake liver enzymes could not be shown. Secondly, recall bias is considered as one of the limitation of FFQ; hence, individuals may be under or overestimated their food intakes. Thirdly, some of nitrate sources were not entered, such as nitrate in soil and water of region.

## Conclusion

The findings indicate that there was a direct relationship between nitrate intake and serum concentration of ALT, but not with the other liver enzymes, including GGT, AST, and ALP.

Given that there is no human study examining the association between nitrate intakes with liver enzymes and there is controversy about results of animal studies, further studies are required to examine the association between dietary nitrate intake and liver functional tests.

## Acknowledgments

This research was funded by the Mashhad University of Medical Sciences. The authors are grateful to all study participants, volunteers, and study personnel.

## Funding

Mashhad University of Medical Sciences (MUMS) supported this study.

## Conflict of interest

The authors declare no competing interests.

## Declarations

Ethics approval and consent to participate

The ethical committee of Mashhad University of Medical Sciences in Mashhad approved the written informed consent (code number: 931188). The written informed consent was signed by all participants before the beginning study.

## Author's contributions

S.Kh and M.G-M designed and conducted the study; Z. D wrote the manuscript and involved in the analysis. M.G.M and G.A.F critically revised the manuscript; S.Kh supervised the study. The final version of the manuscript was approved by all authors.

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