Original Article

Association of Beverage Consumption with Cardio-metabolic Risk Factors and Alanine Transaminase Levels in Children and Adolescents: The CASPIAN-V Study

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Abstract

Background: This study aims to evaluate the association of beverage consumption with cardiometabolic risk factors and alanine transaminase (ALT) levels in children and adolescents.

Materials and Methods: This nationwide study is a part of the fifth survey of a national surveillance program in Iran. Overall, 4200 students, aged 7–18 years, were selected from 30 provinces by multi-stage cluster sampling. In addition to filling in questionnaires, blood sampling and biochemical tests were done. The weekly use of six different beverage types including milk, juice, tea, coffee, soda, and non-alcoholic beer, was documented by interview.

Results: The participation rate was 91.5% (n = 3843), and data of 3733 students were complete for the current study**.** Beverages containing high levels of sugar such as soda and non-alcoholic beer were significantly associated with higher levels of ALT. Model coefficient of regression (SD) was 0.66 (0.31) (*P* value: 0.034). Healthy beverages such as milk and fresh juice and also beverages containing high levels of caffeine did not have significant association with ALT levels (*P* value = 0.32, *P* value = 0.60). Healthy beverages had a significant and inverse relationship with triglycerides (TG) (*P* value = 0.029), total cholesterol (TC) (*P* value = 0.008) and low-density lipoprotein (LDL) (*P* value = 0.008) levels.

Conclusion: This study showed that consuming sugar-sweetened beverages is significantly associated with higher levels of ALT, whereas healthy beverages are associated with a better cardiometabolic profile meaning that consuming healthy beverages leads to lower TG, TC, and LDL levels. The effects of beverages on children's health should be emphasized in health recommendations.

Keywords: Beverages, cardiometabolic risk factors, children and adolescents, liver enzyme

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Introduction

Prevention of non-communicable diseases and their risk factors is a priority for pediatricians. Different underlying factors are considered in this regard. Alanine transaminase (ALT) is a liver enzyme; its rising levels might indicate injuries to

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hepatic cells and therefore it can be used as a proper marker for diagnosing liver disorders.[1] It is also suggested that higher levels of ALT may be independently associated with metabolic disorders such as diabetes.[2] Today, several types of food consumption have increased around the world, but some types of foods such as soft drinks and other sources of added sugar seem to have a higher increase.[3] More than 74% of the Western diet contains added sugar and one of the major sources of this high amount of added sugar is thought to be sugar-sweetened beverages (SSB)s.[4] It has been reported that SSBs are responsible for more than 40% of the added sugar in the population's diet.[5] Fructose is a lipogenic sugar, which is present in processed and industrial beverages all over the world and is responsible for the sweetness of SSBs.[6] Fructose consumption increases the risk of death[7] and some cardiometabolic risk factors.[8] Consuming SSBs could have negative consequences on children's cardiometabolic profiles[9] and is associated with higher body mass index (BMI) levels.[10] Intake of SSBs is also thought to increase waist circumference, raise blood triglycerides (TG), and lower high-density lipoprotein (HDL) values in adolescents.[11,12] It should be mentioned that earlier national studies have found an increase in the prevalence of metabolic syndrome and cardiometabolic risk factors in Iranian adolescents and children.[13]

High levels of sugar intake have been reported to be associated with a variety of liver conditions most notably fatty liver disease.[11,14] Fatty liver disease is one of the most frequent types of liver disease, and it is believed that up to one billion people worldwide are affected by it,[12] and is the demonstration of metabolic syndrome in the liver,[15] even in the pediatric age group.[16] Although fatty liver disease is more frequent in Western countries; it's had a growing trend in other populations.[17] The primary reasons for the increase in the prevalence of fatty liver disease on a global scale are thought to be changes in lifestyle behaviors, including nutrition and physical activity, as well as the rising obesity trend.[16] According to a systematic review, the prevalence of fatty liver disease was reported to be 33.9% in the Iranian population.[18] These statistics are of high importance, considering the fact that fatty liver disease may lead to several more serious liver problems.

The purpose of this study is to analyze the relationship between different types of beverages and blood levels of ALT and cardiometabolic risk factors in a broad group of children and adolescents.

Materials and Methods

This study is a part of the CASPIAN-V study. CASPIAN-V stands for the fifth Childhood and Adolescence Surveillance and Prevention of Adult Non-communicable Disease program, which is a nationwide and school-based surveillance study performed in Iran. Details on the methodology have been presented before and here we briefly report it.

Study population and sampling framework

Students aged 7–18 years coming from both urban and rural areas of all provinces of the country were considered as the study population. They were selected using a multistage, stratified cluster sampling method. We sampled students in each province considering the student's place of residence (urban or rural) and educational degree (primary or secondary) ± with equal sex ratio. We did this in order to obtain the same number of boys and girls in each province. The desired number of samples was obtained using cluster sampling in each province with equal cluster sizes, which were targeted at ten students per cluster, meaning that a total of 10 statistical units (including ten students and their parents) would be considered in each cluster.

In each province of the country, we collected 48 clusters (with ten students in each). This made for 14,400 overall students across the country. Around 4200 one of these 14,400 students, were selected for blood and biochemical testing.

Procedure and measurements

Questionnaires

A set of questionnaires was considered for students and another one for their parents. The student questionnaire was a translated version of the WHO-GSHS (world health organization-Global School-based Student Health Survey). The validity and reliability of questionnaires have been assessed previously.[19] Content validity assessment was approved by an expert pane and all those questions getting a score of more than 0.75 were considered to have appropriate content validity. Cronbach's alpha coefficient for the whole questionnaires was 0.97 and Pearson's correlation coefficient of the test-retest phase was 0.94, which confirmed the reliability of the questionnaires.[20]

The purpose of the study was explained to the eligible students. They understood that the questions are related to their overall health status and health-associated behaviors.

Physical measurements

Using approved checklists, a team of trained healthcare professionals recorded information; Examinations were conducted under standard protocols and using calibrated measures. Weight and height were measured with minimal error with accurate scales. Body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). In order to categorize BMI, WHO growth charts were used.

Beverage consumption

The weekly drinking frequency of six different beverage types including milk, juice, tea, coffee, soda, and non-alcoholic beer was asked among the participants. After that, the beverages were clustered into three categories. The first category was healthy beverages, which included milk and fresh juice. The second category was beverages with high amounts of sugar, which consisted of soda and non-alcoholic beer. The third category was beverages with high amounts of caffeine and theine which included coffee and tea.

Blood sampling

Around 6 mL venous blood sample was collected after 12 hours of overnight fasting from eligible students. All collection tubes were centrifuged at 2500–3000 ×g for 10 minutes. Immediately after that, serum samples were aliquot into 200 μL tubes and stored at −70°C. All of these samples were then transferred by cold chain to Isfahan Mahdieh Laboratory. Using Hitachi Auto Analyzer (Tokyo, Japan), ± Fasting blood glucose, alanine aminotransferase, creatinine, and lipid profile variables were measured enzymatically.

Definitions

Fasting blood sugar ≥100 mg/dl, serum triglyceride (TG) ≥100 mg/dl, total cholesterol (TC) ≥200 mg/dl, low-density lipoprotein (LDL) ≥110 mg/dl were considered abnormal. High-density lipoprotein (HDL) <40 was considered low, HDL >45 was considered acceptable and HDL = 40-45 was considered borderline.[21,22]

The physical activity of the participants was measured by the number of hours of exercise every individual thought they did in a week. This included both the amount of exercise every individual had in physical education classes in school and also the amount of exercise they did on their own.

Beverage classification

The beverages were divided into three categories: Healthy beverages that included milk and fresh juice, beverages containing high amounts of caffeine and theine including coffee and tea, and sugar-sweetened beverages including soda and non-alcoholic beer.

Ethical concerns

Ethical committees and other relevant national regulatory organizations reviewed and approved the study protocols. The study was approved after the full description of its purposes, by the Isfahan University of Medical Sciences Council of Research. Consent was obtained from the children and their families.

Statistical analysis

Data were analyzed using STATA package version 11.0 (Stata Statistical Software: Release 11. StataCorp LP. Package, College Station, TX, USA). A *P* value below 0.05 was considered statistically significant. The multiple linear regression modeling was used to determine the association between ALT and cardiometabolic levels with beverages as dependent and independent variables respectively. The dependent variables were then adjusted by age, gender, FBS, and Physical activity. All statistical measures were estimated using survey data analysis methods.

Results

Overall, 3733 children and adolescents (52.3% boys) completed this study, their mean (SD) age was 12.45 (3.04) years. The mean level of ALT was 7.70 (±3.42). Mean lipid profile indicators consisting of HDL, IDL and TG were 46.24 (±9.98), 153.30 (±27.01), and 86.09 (±39.52) mg/dl, respectively. Mean FBS was measured to be 91.56 (±11.72) mg/dl. The mean BMI was 18.47 (±4.68) kg/m^2.

In this research, we used the analysis of variance method with repeated measures after checking the defaults, to compare the average of quantitative variables in three dependent groups. The basis of work in the analysis of variance (Analysis of Variance) or ANOVA for short, is the analysis of changes or total dispersion according to the factor variable of grouping (Between Group) and error (Error). Of course, in ANOVA with repeated measures, the error dispersion itself is separated into two other components called "within group dispersion" and model error.

This causes the level of model error to decrease. According to this, if there is a dependence between the observations at different levels of a factor, the use of repeated measures ANOVA is superior to the simple ANOVA because the implemented design has less error. Based on the results of the above table and the *P* value column, it is possible to reject the null hypothesis (that is, the equality of the averages of the three groups) for the FBS and physical activity variables. Because the *P* value is smaller than the minimum probability of type 1 error (0.05) and for other variables, the equality of the averages of the three groups is accepted [Table 1].

The frequency of consumption of six different beverage types was asked among the participants. These six beverage types included: milk, juice, soda, non-alcoholic beer, tea, and coffee. As shown in Table 2, tea was the beverage with the highest frequency of consumption, followed by milk and juice, respectively.

Table 1: Characteristics of study participants in groups A, B, C, and total: the CASPIAN-V study

	Group A Mean (SD)	Group B Mean (SD)	Group C Mean (SD)	Total Mean (SD)	*F*	*P*
ALT (unit/l)	8.36 (0.121)	8.37 (0.125)	8.41 (0.135)	8.33 (7.071)	0.879	0.777
FBS (mg/dl)	91.70 (0.207)	91.81 (0.210)	91.66 (0.223)	91.65 (12.11)	1.222	0.027*
TG (mg/dl)	88.49 (0.765)	88.24 (0.779)	87.94 (0.798)	88.04 (45.18)	0.905	0.921
LDL (mg/dl)	90.16 (0.382)	90.17 (0.389)	90.26 (0.406)	90.05 (22.61)	0.977	0.689
HDL (mg/dl)	46.24 (0.167)	46.25 (0.169)	46.25 (0.179)	46.19 (9.98)	0.994	0.501
Age (year)	12.45 (0.051)	12.45 (0.052)	12.48 (0.055)	12.28 (3.16)	0.870	0.637
BMI (kg/m^2)	18.51 (0.080)	18.49 (0.082)	18.51 (0.087)	18.51 (4.71)	1.234	0.285
Physical activity (hours/week)	19.01 (0.127)	18.92 (0.130)	18.96 (0.141)	19.18 (7.73)	61.906	<0.001*

Group A: Milk and Fresh juice. Group B: Tea and Coffee. Group C: Soda and Non-alcoholic beer. *Statistically significant (0.05)

Using factor analysis we categorized the beverage types into 3 categories:

- Healthy beverages, including milk and fresh juice.
- Beverage containing high caffeine and theine, including tea and coffee.
- Beverage containing high sugar, including soda and non-alcoholic beer.

The Linear regression for ALT levels and these three beverage categories for both crude and adjusted models are shown in Table 3. Accordingly, all three beverage groups were substantially related to increased ALT levels in the crude model. After controlling for age, gender, physical activity, and FBS levels, beverages high in sugar were substantially related to higher levels of ALT (*P* value: 0.03). Whereas, consumption of either healthy beverages or beverages with high content of caffeine and theine was not significantly associated with higher levels of ALT (*P* value = 0.32 and 0.60, respectively).

The association between cardiometabolic risk variables and the three beverage types was investigated. As shown in Table 4, only healthy beverages, i.e. milk and fresh juice, had a significant relationship with cardiometabolic indexes. This relationship was inverse, suggesting that higher consumption of healthy beverages was associated with lower cardiometabolic risk. After adjusting the results for gender, age, physical activity, and FBS, the relation between beverage categories and cardiometabolic risk factors was no more significant.

Discussion

Performing this study, we concluded that beverages enriched with sugar or in other words sugar-sweetened beverages such as soda are significantly associated with higher levels of ALT. Since ALT is an indicator for hepatocellular injury and liver disorders we can conclude that SSB consumption can lead to future liver problems and most significantly fatty liver disease. Albeit, it should be noted that ALT levels can predict the presence of fatty liver disease only when two conditions are met, which are the absence of any kind of chronic liver disease such as chronic hepatitis and existing of elements of the metabolic syndrome such as increased BMI.[23] Studying 6370 Japanese subjects, Miyake *et al*.[24] proposed an ALT cutoff level of 25 and 17 U/L for diagnosis of fatty liver disease in men and women respectively. Although abnormal ALT level is associated with fatty liver disease, fatty liver can also be seen in a patient with normal ALT levels, which may be due

Table 2: Frequency (percentage) of consumption of different beverages among participants: the CASPIAN-V study

Variable	Milk	Non- Alcoholic Beer	Soda	Tea	Coffee	Juice	*t*	*P*
Daily1	39.0 (1455)	4.1 (152)	3.1 (116)	51.5 (1922)	5.4 (203)	16.8 (626)	−34.74	<0.0001
Weekly2	44.4 (1656)	13.0 (484)	26.2 (978)	23.9 (892)	11.2 (417)	33.6 (1255)	−35.63	0.00*
Rarely3	10.0 (372)	36.8 (1373)	45.3 (1692)	11.1 (414)	38.9 (1454)	40.7 (1519)	−76.48	0.00*
Never 4	6.5 (241)	44.5 (1663)	25.4 (947)	13.0 (486)	44.2 (1650)	8.2 (307)	−70.92	0.00*
Total	99.8 (3724)	98.4 (3672)	100.0 (3733)	99.5 (3714)	99.8 (3724)	99.3 (3707)	-	-
Missing	0.2 (9)	1.6 (61)	0 (0)	0.5 (19)	0.2 (9)	0.7 (26)	-	-

*Statistically significant. 1: daily consumes that beverage. 2: weekly consumes that beverage. 3: rarely consumes that beverage. 4: never consumes that beverage

Table 3: Linear regression for the association of ALT levels with other variables: the CASPIAN-V study

	Model 1			Model 2		
	β	SE	*P*	β	SE	*P*
Healthy beverage	1.09	0.05	0.00*	−0.27	0.27	0.32
Beverages with high amount of caffeine and theine	1.22	0.06	0.00*	0.14	0.28	0.60
Beverages with high amount of sugar	1.17	0.06	0.00*	0.66	0.31	0.03*

Beta: coefficient of regression. SE: standard error. Model 1: crude model. Model 2: model adjusted by age, sex, physical activity, and FBS. *Statistically significant

Table 4: Association of beverage consumption and cardiometabolic risk factors: the CASPIAN-V study

	Model 1						Model 2					
	TC		TGLDL				TC		TG		LDL	
	β±(SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Healthy beverage	−0.34 (0.15)	0.00	−1.48 (0.04)	0.02	−0.83 (0.03)	0.00	−0.62 (0.50)	0.2	−0.07 (0.62)	0.08	−0.98 (0.58)	0.09
Beverages with high amount of caffeine and theine	0.66 (2.56)	0.69	3.13 (1.62)	0.85	0.59 (1.64)	0.69	0.44 (0.50)	0.38	−1.06 (0.62)	0.91	0.55 (0.57)	0.33
Beverages with high amount of sugar	0.09 (2.56)	0.90	−1.23 (1.61)	0.44	0.4 (1.64)	0.90	−0.22 (0.52)	0.67	0.76 (0.3)	0.10	0.41 (0.60)	0.49

Beta: coefficient of regression. SE: standard error. Model 1: crude model. Model 2: adjusted for age, sex, physical activity, and FBS to developing steatohepatitis or advanced cirrhosis in these patients.[25] Furthermore, it appears that nutritious beverages such as milk and juice, as well as beverages containing significant concentrations of caffeine and theine such as coffee and tea, did not influence ALT levels. This also emphasizes the role of sugar as the main ingredient pertaining to ALT, since we know these beverages do not contain high levels of sugar. In our study, we also found that FBS is positively associated with ALT levels, which is also in line with our previous findings and suggests the undeniable role of sugar as the link between SSBs and liver injury.

Our findings are similar to those of a large portion of studies on this subject. In a large-scale, cross-sectional study among adults a study showed a positive dose-dependent relationship between SSB and ALT levels. They stated that daily consumers of SSBs had a 56% higher risk of developing the fatty liver disease compared to non-consumers.[26] In a study of 31 individuals with fatty liver disease and metabolic syndrome risk factors, it was found that fatty liver disease was associated with a higher intake of soft drinks, but that this association was unrelated to metabolic syndrome risk factors.[27] A meta-analysis of the correlation between artificially sweetened beverages and non-alcoholic fatty liver disease found that SSB consumers had a considerably higher risk of developing the fatty liver disease than those who did not consume SSBs on a regular basis.[28] This study indicated that those who consumed SSBs regularly had a 1.53-fold elevated risk of developing fatty liver disease. In a prospective cohort study among postmenopausal women, it was shown that SSBs had a positive relationship with AST and ALT levels in this gender and age category and even moderate intake of SSBs can induce lipogenesis in the liver.[29] In a 6 months randomized intervention study done by Maersk *et al*.[30] it was reported that sucrose-sweetened beverages rises the accumulation of fat in the liver and since can cause ALT rise and fatty liver disease. Conversely a study by Mollard *et al*.[31] showed that even-though hepatic steatosis is associated with a high amount of fat and fried food intake, it is not directly associated with high-sugar intake.

Studying the association between beverage consumption and cardiometabolic indexes we concluded that healthy beverages have a protective effect on these factors. In other words, increasing the usage of these types of beverages would lower the risk of cardiometabolic disorders. It shall be noted that we did not find a significant relationship between unhealthy beverages such as soda or coffee and cardiometabolic indexes. According to a study by Seferidi *et al*.[9] among 1687 children, SSBs and artificially sweetened beverages (ASBs) were associated with higher blood glucose and TG levels. According to a study by Loh *et al*.[32] SSB consumption among adolescents was related to decreased HDL levels and higher TG, blood glucose, and waist circumference.

In urban China in 2020, Li and colleagues assessed the relationship between the consumption of sugar-sweetened beverages and the risk of metabolic syndrome in children and adolescents. It was found through the evaluation of 7143 children and teenagers that drinking a lot of sugar-sweetened beverages increases the risk of metabolic syndrome and abdominal obesity in kids and teenagers. These results indicate that strict regulations aimed at restricting the consumption of sugar-sweetened beverages may be successful in reducing metabolic syndrome and abdominal obesity.[33] Another similar study was conducted in 2020 by Zhu and others. They analyzed data from 3958 Chinese participants aged 6 to 17 years. It was discovered that participants who consumed the most sugar-sweetened beverages per day (≥201.7 mL/day) had total cholesterol and LDL-C levels that were 0.10 mmol/L and 0.09 mmol/L higher, respectively, than those who did not consume any at all (0 mL/day).[34] These data are consistent with our findings.

A cohort study among more than 2000 Dutch children concluded that consuming more amount of sugar-containing beverages at 1 year of age was associated with higher cardiometabolic risk scores at the age of 6 years, solely in boys.[35] Another study by Ambrosini *et al*.[36] presented a similar finding in both genders among adolescents. Using a healthy beverage index (HBI) score, Duffy and Davy stated that healthy beverage consumption would lead to a more favorable lipid and cardiometabolic profile.[37] Research on 4880 children revealed that SSB consumption is associated not only with a poorer lipid profile but also with elevated levels of inflammatory indicators such as C-reactive protein (CRP).[38]

The most significant advantages of this study are its large sample size as well as the fact that it was carried out in each and every one of the country's provinces. One of the limitations of this study is the absence of abdominal HRCT scan images or abdominal sonography regarding the diagnosis of fatty liver disease. Another limitation of this study is that it did not consider the association of demographic status with the participant's diet.

Conclusions

High-sugar beverages are correlated with an increase in ALT levels and may consequently have a significant influence on the development of non-alcoholic fatty liver disease and other liver-related diseases. The use of healthy beverages, as well as beverages with high concentrations of caffeine and theine, is not substantially associated with an increase in ALT levels. Healthy beverages have an inverse relationship with LDL, TC, and TG whereas, unhealthy beverages do not have a significant relationship with them. According to our findings, we suggest that proper policies are made and appropriate actions are undertaken to reduce consuming unhealthy beverages.

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Authors' contribution

MK, AS and RK conceptualized the study in accordance with all authors who contributed in conducting the study, drafting the manuscript, and leading the process of revising the manuscript for submission. RH, MEM, MQ and MN contributed in conducting the study, drafting the manuscript, and its revision. ES and MM were responsible for the statistical methods, analysis, and results section. The final manuscript which was submitted was agreed upon by all the authors. No funds were received or distributed to anyone to produce this manuscript.

Ethics approval and consent to participate

Study protocols were reviewed and approved by ethical committees and other relevant national regulatory organizations. The Research and Ethics Council of Isfahan University of Medical Sciences approved the study (Project Number: 194049). Informed consent were obtained from children and their parents.

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Conflicts of interest

There are no conflicts of interest.

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