

Original Article

Increased dietary zinc and vitamin B-2 is associated with increased alanine aminotransferase in Taiwanese adolescents

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Background and Objectives: Alanine aminotransferase (ALT) is generally used for evaluating liver function, and its concentrations are closely associated with sex and nutritional status. This study investigates the relationships between dietary components and serum ALT activity in Taiwanese adolescents. **Methods and Study Design:** Data were collected from 1,941 adolescents aged 13-18 years who participated in the fourth National Nutrition and Health Survey in Taiwan (2010-2011, adolescents). **Results:** The mean age was 15.3±0.1 y (15.3±0.1 y for boys and 15.2±0.1 y for girls). Mean serum ALT was 14.8±13.3 U/L (17.7±16.3 U/L for boys and 12.1±8.7 U/L for girls; $p < 0.001$). Multivariate analysis revealed that, among girls, a single-unit increase in dietary zinc was associated with 1.12- and 1.11-fold increases in risk for increased serum ALT tertile 2 (T2) and T3, respectively, compared with T1; and a single-unit increase in vitamin B-2 intake increased risk by 1.71- and 1.54-fold, respectively. Further analysis revealed that the risk increase for boys and girls who consumed the highest amounts of dietary zinc and vitamin B-2 (T3) was 1.97- and 2.62-fold, respectively; they were also more likely to have higher serum ALT (>11 U/L for boys and >9 U/L for girls) than those of the reference (presented as zinc T1 and vitamin B-1 T1). **Conclusions:** Increased dietary zinc and vitamin B-2 intake is associated with higher serum ALT in adolescents.

Key Words: dietary zinc, dietary vitamin B-2, serum alanine aminotransferase, hepatic injury, adolescents

INTRODUCTION

The body mass index (BMI) is used for predicting steatosis, nonalcoholic steatohepatitis (NASH), and higher serum alanine aminotransferase (ALT).¹ Dietary control and lifestyle changes are considered effective and safe approaches for treating steatosis and NASH,² but a low-energy diet (LED) may also lead to transient hepatic injury.^{3,4} Gasteyer and colleagues have reported that LED-induced weight reduction triggered a transient increase in serum ALT concentrations in obese women, but not in obese men.^{3,4} Why women are more susceptible to LED-induced transient liver injury remains unclear. Ganz et al investigated how sex differences influence diet-induced nonalcoholic fatty liver disease (NAFLD) in mice fed based on a high-fat diet enriched with fructose and sucrose, and observed preferential NASH and inflammatory activation in male mice, whereas steatosis without inflammation was noted in the female mice.⁵ A study that investigated food preferences based on gender found that boys preferred meat, fish, and poultry foods, whereas girls preferred fruits and vegetables.⁶ Meat, particularly

red meat, is a rich source of protein, fat, and trace elements (e.g., iron, zinc, and copper). Trace elements act as either pro-oxidants or antioxidants. For example, certain antioxidant enzymes (e.g., catalase and superoxide dismutase) require iron, zinc, and copper as cofactors. However, free-transition metal ions are also potent pro-oxidants. By contrast, fruits and vegetables contain high levels of vitamins with antioxidant properties (e.g., vitamin C and β -carotene), which are vital for preventing the progression of simple steatosis into NASH.⁷ Women also have a higher body fat percentage and greater lipid perox-

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idation levels;⁸ hence, women may require more foods rich in antioxidants for preventing hepatic injury when losing weight.

Nearly half of all children in Taiwan are currently either overweight or obese.⁹ Wu et al investigated the nutritional intake profiles of Taiwanese children aged 6-12 years, and noted an insufficient intake of vitamins (B complex and vitamin A) and iron in a higher proportion of girls compared with boys.¹⁰ Vitamin B complex plays a critical role in liver function.¹¹ The relationship between dietary components and liver function in adolescents remains poorly understood. We hypothesized that sex-related differences in food intake (both micro- and macronutrients) may influence liver function. Thus, this study investigates the relationships between dietary components and serum ALT activity (as an indicator of liver function) in Taiwanese adolescents by using data from the Nutrition and Health Survey in Taiwan (NAHSIT 2010-2011, adolescents).

METHODS

Study design

The fourth national NAHSIT was funded by the Food and Drug Administration, Ministry of Health and Welfare, Taiwan, for the continued evaluation of the health and nutritional status of the Taiwan population. This study analyzed data on adolescents aged ≥ 13 -18 y collected through the fourth NAHSIT. A multistage, stratified, clustered sampling scheme was used to recruit participants. Details of the study design have been published.^{10,12,13} In brief, 5 sampling strata were selected based on geographical location and population density. An additional mountainous stratum was included for junior high school children (aged 12-16 years). Multistage probability sampling proportional to size was conducted to select 8 schools from each stratum; 36 junior high schools and 20 senior high schools were identified. A random sample of 54 (9 students/sex/grade) junior and 60 (10 students/sex/grade) senior high school students was selected from each school. The response rate for the questionnaires was 46% and 42%, respectively. Only children who completed the questionnaires qualified for participation in the anthropometric examination (response rate of 87% and 92%, respectively). The survey respondents comprised 3,228 adolescents (1,952 junior and 1,276 senior high school students). Informed parental written consent was obtained before participant enrolment in the NAHSIT study. The NAHSIT study was approved by the Research Ethics Committee of Academia Sinica (EC100031). This study used a databank that excluded the private information of individual respondents, and was approved by Taipei Medical University (201210005).

Sample inclusion and exclusion criteria

Data from 1,941 adolescent respondents (963 boys and 978 girls) were included in the analysis. The exclusion criteria were as follows: (1) Respondents with missing data on serum ALT, anthropometrics, or 24-hour dietary recall ($n=1,244$); (2) total energy intake $\geq 5,000$ kcal/d or ≤ 500 kcal/d ($n=5$); and (3) a history of hepatitis, hepatocarcinoma, diabetes, nephritis, autoimmune disease, or cancer ($n=38$).

Data collection

Information on family health history (self-reported), 24-hour dietary recall, and factors associated with personal behaviour were obtained using structural questionnaires with a standardized protocol for the interview. Dietary intake was estimated by referring to the 24-hour dietary recall, which included measurements of foods consumed regularly in each household individual dietary recall, and their validation as obtained from food models.¹⁰ Dietary data on total caloric intake, carbohydrates (CHOs), protein, fats and oils, fruits, vegetables, water- and fat-soluble vitamins, and trace elements were obtained from the 24-hour dietary recall. The details regarding data collection and data analysis have been published elsewhere.^{10,12,13}

Laboratory measurements

Biochemical data were obtained from 8-hour fasting blood samples. Peripheral venous blood samples were collected in tubes containing EDTA, and were centrifuged at 4°C before the serum was stored at -80°C until analysis. ALT and aspartate aminotransferase concentrations were measured using a Beckman Coulter LX-20 autoanalyzer (Fullerton, CA, USA).

Statistical analyses

Statistical analyses were performed using SAS software (Version 9.22; SAS Institute, Cary, NC, USA). Categorical data are presented numerically (percentage), whereas continuous data are presented as the mean (standard deviation (SD)). Serum ALT concentrations were categorized into tertiles (Ts) by sex: 11 U/L and 16 U/L for boys, and 9 U/L and 12 U/L for girls. Trend testing was conducted by employing either an ordinal chi-squared test or a general linear regression model for continuous data (Table 2). Multivariable logistic regression models were used to estimate the odds ratio (OR) for the dependent variable (serum ALT) and independent variables (age, sex, BMI, and levels of protein, vitamin B-2, niacin, vitamin B-6, vitamin C, iron, zinc, magnesium, potassium, and phosphorus), in addition to the 95% confidence interval (CI) (Table 3). A binary logistic model was employed for further analysis on the relationships of dietary zinc and vitamin B-2 with the risk of elevated ALT levels. As shown in Figure 1, a binary logistic regression model was used to estimate the OR of dependent ALT T2 plus T3 compared with ALT T1 (reference). The residual method was used to adjust all nutrient intake levels based on total energy;¹⁴ $p < 0.05$ was considered significant.

RESULTS

Participant characteristics

Data from 1,941 adolescents (963 boys and 978 girls) were included in this study. The mean age was 15.3 ± 0.1 yr (15.3 ± 0.1 yr for boys and 15.2 ± 0.1 yr for girls) (Table 1). The mean BMI was 21.4 ± 0.2 kg/m² (21.9 ± 0.2 kg/m² for boys and 21.0 ± 0.1 kg/m² for girls, $p < 0.001$) (Table 1). The mean ALT was 14.8 ± 13.3 U/L (17.7 ± 16.3 U/L for boys and 12.1 ± 8.7 U/L for girls, $p < 0.001$) (Table 1). Overall, the boys weighed more and had higher serum ALT levels compared with their female counterparts ($p < 0.001$ for both sexes) (Table 1).

Table 1. Baseline characteristics of the sample population (N=1,941)

Variable [†]	Sex		p value*
	Boys	Girls	
Number (%)	963 (49.6)	978 (50.4)	
Age (yr)	15.3 (0.10)	15.2 (0.10)	0.262
Waist-to-hip ratio	0.82 (0.06)	0.80 (0.05)	<0.0001
Body mass index (kg/m ²)	21.9 (0.20)	21.0 (0.10)	<0.0001
Serum aspartate aminotransferase (U/L)	21.4 (0.30)	17.8 (0.20)	<0.0001
Serum alanine aminotransferase (U/L)	17.7 (16.3)	12.1 (8.70)	<0.0001

[†]Continuous data are presented as the mean (standard deviation).

*Between-group differences were analyzed by conducting a two-sample *t* test.

Table 2. Nutritional factor characteristics according to serum alanine aminotransferase tertiles among Taiwanese adolescents (N=1,941)

	Serum ALT, Tertile [§]			p for trends*
	1	2	3	
Number	533	757	651	
Age (y)	14.9 (1.82)	15.1 (1.87)	15.6 (1.95)	<0.0001
BMI (kg/m ²) [†]	19.9 (2.88)	20.5 (3.16)	23.8 (5.20)	<0.0001
24-hour dietary intake/d [‡]				
Carbohydrates (g/d)	308 (2.64)	302 (2.15)	300 (2.43)	0.040
Simple sugars	57.2 (2.20)	52.8 (1.80)	52.0 (2.02)	0.089
Complex sugars	253 (2.77)	252 (2.26)	250 (2.55)	0.444
Fat (g/d)	87.7 (1.03)	87.7 (1.03)	89.0 (0.95)	0.387
Protein (g/d)	85.6 (0.97)	89.9 (0.79)	91.8 (0.89)	<0.0001
Vitamin B-1 (mg/d)	1.39 (0.03)	1.45 (0.02)	1.49 (0.03)	0.025
Vitamin B-2 (mg/d)	1.35 (0.04)	1.44 (0.03)	1.45 (0.04)	0.004
Niacin (mg/d)	20.0 (0.45)	21.0 (0.37)	21.8 (0.42)	0.005
Vitamin B-6 (mg/d)	1.65 (0.04)	1.82 (0.03)	1.83 (0.03)	<0.001
Vitamin B-12 (µg/d)	4.61 (0.30)	4.64 (0.24)	5.53 (0.28)	0.030
Vitamin C (mg/d)	104 (5.08)	113 (4.14)	130 (4.68)	<0.001
Vitamin A (µg RE/d)	698 (82.1)	703 (66.9)	821 (75.6)	0.289
Vitamin D (µg/d)	5.20 (0.29)	5.34 (0.24)	5.85 (0.27)	0.115
Vitamin E (mg/d)	8.69 (0.21)	9.03 (0.17)	9.14 (0.19)	0.133
Iron (mg/d)	16.1 (0.38)	17.3 (0.31)	17.6 (0.35)	0.005
Zinc (mg/d)	10.9 (0.18)	11.6 (0.14)	12.1 (0.16)	<0.0001
Calcium (mg/d)	467 (12.9)	510 (10.5)	519 (11.85)	0.004
Magnesium (mg/d)	241 (3.64)	257 (2.97)	261 (3.56)	<0.001
Potassium (mg/d)	2213 (34)	2383 (28)	2450 (31)	<0.0001
Phosphorus (mg/d)	1213 (15)	1284 (12)	1294 (13)	<0.0001

[†]The body mass index (BMI) was calculated as the mass (kg)/[height (m)]².

[‡]Nutrient intake was obtained by adjusting for energy through the residual method.

[§]Serum alanine aminotransferase (ALT) tertiles by sex: 11 U/L and 16 U/L for boys, and 9 U/L and 12 U/L for girls.

*The trend test was conducted using a general linear model for continuous variables.

Associations between serum ALT tertiles and nutritional factors

The baseline characteristics of the nutritional factors of Taiwanese adolescents are listed by serum ALT tertiles in Table 2. The table shows a positive trend of serum ALT tertiles for age and BMI ($p < 0.0001$ for both variables) (Table 2). Moreover, dietary components such as protein, CHOs, vitamin B complex, vitamin C, divalent metals, and electrolytes were found to be positively associated with the ALT tertiles ($p < 0.05$ for all components). Conversely, no association was found between higher serum ALT tertiles and dietary fat or fat-soluble vitamins (i.e., vitamins A, D, and E) (Table 2).

The results of univariate analysis revealed that a single-unit increase in factors such as age; BMI; protein; vitamins B-2, B-3, B-6, and C; divalent metals (i.e., iron and zinc); and electrolytes (i.e., potassium, phosphorus, and magnesium) was associated with higher ALT tertiles

(data not shown). In boys, multivariate analysis adjusted for age, BMI, and nutrients revealed that a single-unit increase in BMI, age, and zinc intake was associated with higher ALT levels, specified as follows; BMI (OR: 1.42, 95% CI: 1.34-1.51), age (OR: 1.15, 95% CI: 1.03-1.27), and zinc (OR: 1.00, 95% CI: 1.00-1.07) for boys with the highest ALT (T3) compared with those with the lowest ALT (T1) (multivariate model: boys) (Table 3). Similar analytical results were obtained for girls; an elevation in ALT levels (ALT T3 compared against T1) resulting from its association with age (OR: 1.16, 95% CI: 1.05-1.27) and BMI (OR: 1.21, 95% CI: 1.14-1.28) (multivariate model: girls) (Table 3). In addition, the serum ALT levels appeared to rise in conjunction with dietary zinc and vitamin B-2 levels for girls; specifically, the effect of a single-unit increase in dietary zinc on ALT T2 and T3 (compared against T1) was 1.12- and 1.11-fold, respectively, whereas in vitamin B-2, it was 1.71- and

Table 3. Adjusted odds ratio and 95% confidence intervals of dietary components according to the tertile groups of serum alanine aminotransferase levels

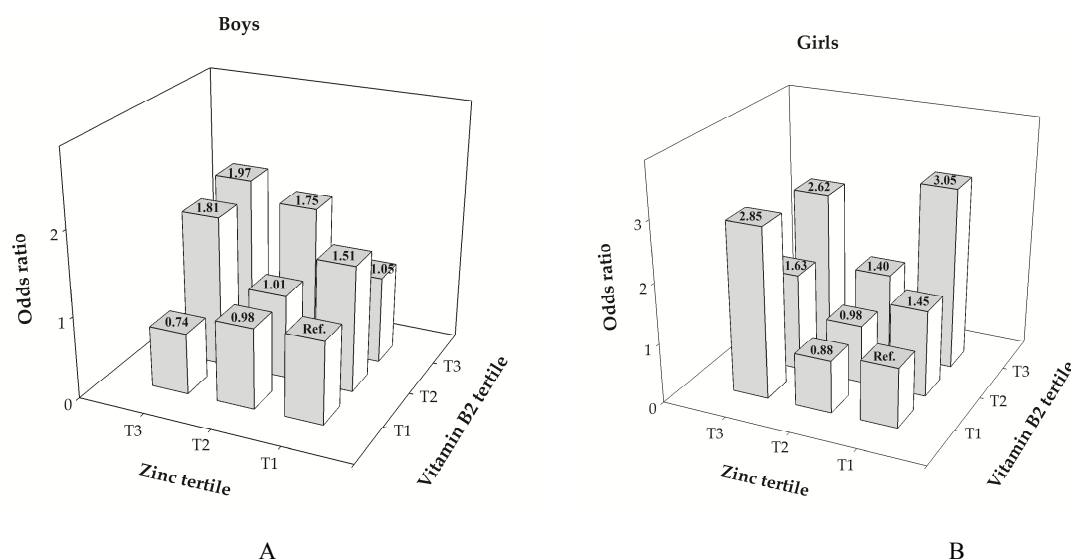
	Boys, ALT tertiles ^{†,‡}		Girls, ALT tertiles ^{†,§}	
	T2 [¶]	T3 [¶]	T2 [¶]	T3 [¶]
Age (yr)	1.07 (0.97-1.17)	1.15 (1.03-1.27)	1.04 (0.95-1.14)	1.16 (1.05-1.27)
BMI (kg/m ²)	1.14 (1.08-1.21)	1.42 (1.34-1.51)	1.08 (1.02-1.14)	1.21 (1.14-1.28)
24-hour dietary intake/d				
Protein (g/d)	1.01 (0.99-1.02)	1.00 (0.99-1.02)	0.98 (0.97-1.00)	0.99 (0.98-1.01)
Vitamin B-2 (mg/d)			1.71 (1.10-2.64)	1.54 (0.98-2.41)
Niacin (mg/d)	1.01 (0.99-1.03)	1.01 (0.99-1.03)		
Vitamin B-6 (mg/d)			1.09 (0.77-1.53)	1.35 (0.96-1.53)
Vitamin C (mg/d)	1.00(0.998-1.002)	1.00(0.999-1.003)		
Iron (mg/d)				
All	0.98 (0.95-1.02)	0.99 (0.95-1.03)	1.02 (0.99-1.05)	1.01 (0.98-1.04)
Nonheme iron (mg/d)	1.04 (0.98-1.09)	1.03 (0.97-1.09)		
Magnesium (mg/d)	1.00 (0.997-1.003)	1.00 (0.996-1.003)	1.00 (0.998-1.005)	1.00 (0.997-1.005)
Zinc (mg/d)	0.98 (0.93-1.04)	1.00 (1.00-1.07)	1.12 (1.03-1.23)	1.11 (1.01-1.21)

[†]Serum alanine aminotransferase (ALT) tertiles by sex: 11 U/L and 16 U/L for boys, and 9 U/L and 12 U/L for girls.

[‡]The multivariate model was adjusted for age, body mass index (BMI), and nutrients (i.e., protein, niacin, vitamin C, iron, nonheme iron, magnesium, zinc, phosphorus, and potassium).

[§]The multivariate model was adjusted for age, BMI, and nutrients (i.e., protein, vitamin B-2, vitamin B-6, iron, magnesium, zinc, phosphorus, and potassium).

[¶]T1 was reference.

**Figure 1.** Age and body mass index: Adjusted odds ratio and 95% confidence intervals for dietary zinc and vitamin B-2 on serum alanine aminotransferase tertiles in boys (A) and girls (B).

1.54-fold, respectively (multivariate model: girls) (Table 3).

Dietary zinc and vitamin B-2 inducing a rise in serum ALT

A categorical logistic model was employed to further classify the relationships of dietary zinc and vitamin B-2 with the risk of serum ALT elevation. Figure 1 shows the adjusted ORs for the risk of serum ALT elevation associated with dietary zinc and vitamin B-2 intake levels. In both boys (A) and girls (B), only adolescents with intake results in the highest zinc tertile (T3) and vitamin B-2 tertile (T3) were affected, with a 1.97- (95% CI, 1.22–3.18) and 2.62-fold (95% CI, 1.58–4.35) increase, respectively. The affected adolescents were more likely to have higher serum ALT levels (>11 U/L for boys and >9 U/L for girls) compared with the reference (i.e., represented by zinc T1 and vitamin B-1 T1) (Figure 1).

DISCUSSION

Based on our research, this cross-sectional, population-based study is the first to investigate associations between food intake and liver function in Taiwanese (i.e., mainly ethnic Chinese) adolescents. Our findings suggest a positive causal relationship between the dietary intake of zinc and vitamin B-2 and serum ALT levels in adolescents. However, the reason for this increased risk is unclear. Riboflavin is the precursor for the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Flavoproteins are enzymes that require FMN or FAD as cofactors; they act as electron carriers and play a role in oxidation-reduction (redox) reactions in cells. In addition, they function as prosthetic groups in the first oxidative step of the fatty acid cycle. Several flavoproteins also contain metal ions. Mallik et al found that zinc and several other cations (e.g., cadmium, copper, and iron) inhibit FMN phosphatase activity.¹⁵ FMN Phosphatase is

the enzyme that releases riboflavin from dietary FMN coenzyme forms. Long-term FMN phosphatase suppression may lead to riboflavin deficiency and metabolic failure, raising the likelihood of hepatic injury. However, riboflavin can also regulate the metabolism of divalent metals (e.g., iron, zinc, and copper).¹⁶ Animal studies have found that riboflavin supplementation either enhances zinc absorption⁸ or lowers hepatic zinc concentrations.¹⁷ Furthermore, Adekan and Thurnham indicated that a riboflavin deficiency reduced total iron absorption and inhibited the release of liver ferritin into the peripheral blood, resulting in anaemia in rats.^{16,18} Overall, these studies have posited that riboflavin may regulate the absorption and sequestration of divalent metals such as zinc.

Although in the present study no association was found between macronutrient intake and serum ALT elevation, related studies have reported that macronutrient modifications, especially CHOs, affected serum ALT levels.¹⁹⁻²¹ A hypoenergy diet restricting CHOs but not fat led to lower serum ALT levels in adults with insulin resistance.¹⁹ By contrast, serum ALT activity increased significantly when healthy men adhered to an isocaloric diet high in CHOs, but with normal fat content.²⁰ However, Rodriguez-Hernandez investigated the effects of a low-CHO, low-fat diet on serum ALT activity in obese women with NAFLD, and reported that decreased ALT levels were related to body-weight reduction, irrespective of the type of diet.²² Additional evidence suggests that elevated serum ALT levels can be explained not with the amount of CHOs consumed, but with the quality of the CHO sources in the diet.²¹ For example, CHO sources that either affect blood glucose levels (e.g., foods with a low glycemic index or low glycemic load)²¹ or lipid deposition in the liver (e.g., sucrose or fructose)²³ can affect serum ALT levels. In addition, one study reported that a diet low in animal protein but high in vegetables improved liver function in healthy participants.²⁴ Although animal studies have indicated a close relationship between high-fat diets and liver steatosis, conflicting results have been obtained on the effect of dietary fat restrictions in human studies.¹⁹⁻²¹

The findings of the present study revealed that dietary zinc and vitamin B-2 were independently associated with serum ALT activity in adolescents. Compared with boys, girls seemed more susceptible to elevated ALT levels resulting from a high intake of vitamin B-2 and zinc. The reason for this sex difference is unclear. Vitamin B-2 is water-soluble, and is considered relatively nontoxic compared with fat-soluble vitamins. The average intake of vitamin B-2 (1.57 (0.04) mg for boys and 1.28 (0.03) mg for girls) in our study was comparable with Taiwanese guidelines. Zinc is involved in a wide range of physiological functions. For example, over 100 enzymes involved in catalytic function also contain zinc. In adolescents, zinc is vital for sexual maturation and reproduction. Compared with their female counterparts, adolescent boys require higher levels of zinc because sperm and the prostate gland require zinc to function properly. Conversely, studies have suggested that girls, not boys, are at high risk for inadequate zinc intake.^{25,26} In the present study, the mean dietary zinc intake was 13.7 (6.6) mg/d for boys, and 9.6 (4.8) mg/d for girls, which was slightly lower compared with the Taiwanese guidelines for adolescents

aged 13-18 years (15 mg/d for boys and 12 mg/d for girls). The Taiwan dietary reference intake for zinc appears to be higher compared with U.S. guidelines. The U.S. Recommended Dietary Allowance for zinc is 8 mg/d for adolescents aged approximately 13 years, and 11 mg/d for boys and 9 mg/d for girls aged 14-18 years. The average zinc intake in the United States for boys and girls aged 11-18 years was 12 mg/d and 8 mg/d, respectively, according to the third U.S. National Health and Nutrition Examination Survey (1988-1994).²⁵ This is similar to the findings of a recent report on 1311 Colombian adolescents aged 11-16 years, which indicated an average zinc intake of 10.8 mg/d for boys and 8.7 mg/d for girls.²⁷ Compared with the U.S. and Colombian studies, Taiwanese adolescents appear to consume a greater amount of dietary zinc. Zinc finger proteins are those that contain zinc-binding repeats. In eukaryotes, zinc finger proteins are vital for DNA recognition, RNA packaging, and transcriptional activation. However, a recent study indicated that zinc finger protein 267 is highly expressed in patients with NAFLD, and the authors found that zinc finger protein 267 acts as a profibrogenic factor in NAFLD.²⁸

The early onset of childhood NAFLD in ethnic Chinese children and adolescents is of particular concern.²⁹ A recent investigation on NAFLD prevalence in Chinese school-aged children and adolescents from the Yangtze River Delta region indicated that NAFLD prevalence was strongly associated with body shape, especially for those with abdominal obesity (12.9%) and mixed obesity (44.8%).²⁹ Excess energy intake is frequently associated with NAFLD, but the dietary patterns linked with NAFLD onset and progression remain unclear. The food consumption patterns of adolescents are of particular concern because of the greater energy and nutrients demands required by this vulnerable group compared with adults. At present, a detailed analysis on the dietary intake of Taiwanese adolescents is ongoing and is not yet publically available. According to an earlier NAHSIT survey on children and elderly people (NAHSIT, 2000-2001), the major foods containing vitamin B-2 that are consumed by Taiwanese children are dairy products (0.33 mg), pork (0.16 mg), eggs (0.13 mg), soft drinks (0.1 mg), rice (0.08 mg), and chicken (0.08 mg).^{10,30} The major sources of vitamin B-2 consumed by the elderly Taiwanese population are dairy products (0.52 mg), pork (0.12 mg), wheat and flour products (0.11 mg), fresh fruits (0.1 mg), and dark green and yellow vegetables (0.08 mg).³¹ In addition to dietary intake, another study investigated the effects of vitamin B complex on the risk prediction of all-cause mortality in the elderly Taiwanese population.³² Huang et al reported that the highest tertile of vitamin B-2 intake, after adjusting for dietary diversity, was found to be associated with a 40% increase in the mortality rate of the elderly Taiwanese population.³² However, a higher intake of vitamin B-1 and B-6 was associated with a lower mortality risk for up to 10 years. Foods containing vitamin B-2 consumed by Taiwanese adolescents may belong to numerous protein-rich food groups and, to a lesser extent, vegetables or grains. Protein-rich foods such as seafood and meat are also rich in zinc. Because dietary zinc data are unavailable for research on Taiwanese children as well as the elderly

population, it is difficult to determine whether these 2 groups eat the same foods that contain these nutrients (i.e., zinc and vitamin B-2). Future research focusing on dietary patterns is necessary to clarify the consumption patterns of foods containing both zinc and vitamin B-2. Understanding the effects of dietary patterns on hepatic well-being is critical because food consumption patterns can act as modifiable factors for NAFLD prevention.

Our study had several limitations. First, the exclusion criterion for patients with hepatic disease (e.g., hepatitis viral infection) was based on a self-reported health history, not on clinical data (e.g., hepatitis surface antigen). Second, our study was confined because of the cross-sectional design. In order to establish causality for dietary zinc and vitamin B-2 intake with ALT activity, longitudinal studies are required to determine if changes in zinc/vitamin B-2 intake over time can be used to predict liver disease progression. Third, dietary records spanning only 24 hours might be too brief for use for obtaining reliable data on dietary intake. However, because of wide variations in geographical location and manpower limitations, our NAHSIT questionnaire for dietary intake was restricted to 24 hours. Henríquez-Sánchez et al conducted a MEDLINE and EMBASE literature review of 5,746 vitamin studies, and reported the Food Frequency Questionnaire is suitable for assessing vitamin intake with correlation coefficients with a range of 0.43–0.67 for the 24-hour recall reference.³³

Conclusion

Because of the rapid increase in NAFLD prevalence among children and adults, understanding the link between diet and hepatic health may help to prevent the onset and progression of NAFLD.

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AUTHOR DISCLOSURES

The authors declare no competing interests.

REFERENCES

1. Welsh JA, Karpen S, Vos MB. Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. *J Pediatr*. 2013;162:496-500. doi: 10.1016/j.jpeds.2012.08.043.
2. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. *Am J Clin Nutr*. 2007;86:285-300.
3. Gasteyerger C, Larsen TM, Vercauysse F, Astrup A. Effect of a dietary-induced weight loss on liver enzymes in obese subjects. *Am J Clin Nutr*. 2008;87:1141-7.
4. Friis R, Vaziri ND, Akbarpour F, Afrasiabi A. Effect of rapid weight loss with supplemented fasting on liver tests. *J Clin Gastroenterol*. 1987;9:204-7. doi: 10.1097/00004836-198704000-00019.
5. Ganz M, Csak T, Szabo G. High fat diet feeding results in gender specific steatohepatitis and inflammasome activation. *World J Gastroenterol*. 2014;20:8525-34. doi: 10.3748/wjg.v20.i26.8525.
6. Caine-Bish NL, Scheule B. Gender differences in food preferences of school-aged children and adolescents. *World J Gastroenterol*. 2009;79:532-40. doi: 10.1111/j.1746-1561.2009.00445.x
7. Ued Fda V, Weffort VR. Antioxidant vitamins in the context of nonalcoholic fatty liver disease in obese children and adolescents. *Rev Paul Pediatr*. 2013;31:523-30. doi: 10.1590/S0103-05822013000400016.
8. Agte VV, Paknikar KM, Chiplonkar SA. Effect of riboflavin supplementation on zinc and iron absorption and growth performance in mice. *Biol Trace Elem Res*. 1998;65:109-15. doi: 10.1007/BF02784263.
9. Chang JS, Bai CH, Huang ZC, Eddy O, Chao KC, Chang CC, Chiou HY. Interleukin 10 and clustering of metabolic syndrome components in pediatrics. *Eur J Clin Invest*. 2014;44:384-94. doi: 10.1111/eci.12247.
10. Wu SJ, Pan WH, Yeh NH, Chang HY. Dietary nutrient intake and major food sources: the Nutrition and Health Survey of Taiwan Elementary School Children 2001-2002. *Asia Pac J Clin Nutr*. 2007;16:518-33. doi: 10.6133/apjcn.2007.16.s2.03.
11. Baker H, Frank O, Ziffer H, Goldfarb S, Leevy CM, Sobotka H. Effect of hepatic disease on liver B-complex vitamin titers. *Am J Clin Nutr*. 1964;14:1-6.
12. Huang YC, Lee MS, Pan WH, Wahlqvist ML. Validation of a simplified food frequency questionnaire as used in the Nutrition and Health Survey in Taiwan (NAHSIT) for the elderly. *Asia Pac J Clin Nutr*. 2011;20:134-40. doi: 10.1111/j.1467-789X.2007.00434.x.
13. Pan WH, Hung YT, Shaw NS, Lin W, Lee SD, Chiu CF et al. Elderly Nutrition and Health Survey in Taiwan (1999-2000): research design, methodology and content. *Asia Pac J Clin Nutr*. 2005;14:203-10.
14. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*. 1986;124:17-27.
15. Mallik S, Dey M, Dutta M, Ghosh AK, Bandyopadhyay D. Flavin Mononucleotide phosphatase from goat liver: A possible target for divalent heavy metal cations. *Int J Pharm Pharm Sci*. 2014;6:s708-s14.
16. Powers HJ. Riboflavin (vitamin B-2) and health. *Am J Clin Nutr*. 2003;77:1352-60.
17. Gralak M, Debski B, Lewicka A, Bertrand J, Ktos A, Piastowska-Ciesielska AW, Stryczek, AB, Morka, A. Influence of riboflavin supplementation on liver trace element concentration in trained rats fed low protein diet. *Probl Hig Epidemiol*. 2013;94:839-42.
18. Adelekan DA, Thurnham DI. The influence of riboflavin deficiency on absorption and liver storage of iron in the growing rat. *Brit J Nutr*. 1986;56:171-9. doi: 10.1079/BJN.19860097.
19. Ryan MC, Abbasi F, Lamendola C, Carter S, McLaughlin TL. Serum alanine aminotransferase levels decrease further with carbohydrate than fat restriction in insulin-resistant adults. *Diabetes care*. 2007;30:1075-80. doi: 10.2337/dc06-2169.
20. Purkins L, Love ER, Eve MD, Wooldridge CL, Cowan C, Smart TS, Johnson PJ, Rapeport, W G. The influence of diet

- upon liver function tests and serum lipids in healthy male volunteers resident in a Phase I unit. *Brit J Clin Pharmacol.* 2004;57:199-208.
21. Ramon-Krauel M, Salsberg SL, Ebbeling CB, Voss SD, Mulkern RV, Apura MM, Cooke EA, Sarao K, Jonas MM, Ludwig DS. A low-glycemic-load versus low-fat diet in the treatment of fatty liver in obese children. *Child Obes.* 2013; 9:252-60. doi: 10.1089/chi.2013.0022.
 22. Rodriguez-Hernandez H, Cervantes-Huerta M, Rodriguez-Moran M, Guerrero-Romero F. Decrease of aminotransferase levels in obese women is related to body weight reduction, irrespective of type of diet. *Ann Hepatol.* 2011;10:486-92.
 23. Porikos KP, Van Itallie TB. Diet-induced changes in serum transaminase and triglyceride levels in healthy adult men. Role of sucrose and excess calories. *Am J Med.* 1983;75: 624-30. doi: 10.1016/0002-9343(83)90444-8.
 24. Iwamoto M, Yagi K, Yazumi K, Komine A, Shirouchi B, Sato M. Eating a healthy lunch improves serum alanine aminotransferase activity. *Lipids Health Dis.* 2013;12:134. doi: 10.1186/1476-511X-12-134.
 25. Briefel RR, Bialostosky K, Kennedy-Stephenson J, McDowell MA, Ervin RB, Wright JD. Zinc intake of the U.S. population: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *J Nutr.* 2000;130: 1367S-73S.
 26. Johnson RK, Johnson DG, Wang MQ, Smiciklas-Wright H, Guthrie HA. Characterizing nutrient intakes of adolescents by sociodemographic factors. *J Adolesc Health.* 1994;15: 149-54. doi: 10.1016/1054-139X(94)90542-8.
 27. Suarez-Ortegon MF, Ordenez-Betancourth JE, Aguilar-de Plata C. Dietary zinc intake is inversely associated to metabolic syndrome in male but not in female urban adolescents. *Am J Hum Biol.* 2013;25:550-4. doi: 10.1002/ajhb.22408.
 28. Schnabl B, Czech B, Valletta D, Weiss TS, Kirovski G, Hellerbrand C. Increased expression of zinc finger protein 267 in non-alcoholic fatty liver disease. *Int J Clin Exp Pathol.* 2011;4:661-6.
 29. Zhang X, Wan Y, Zhang S, Liping L, Chen Z, Liu H, Jiang X, Luo K, Cai W. Nonalcoholic fatty liver disease prevalence in urban school-aged children and adolescents from the Yangtze River delta region: a cross-sectional study. *Asia Pac J Clin Nutr.* 2015;24:281-8. doi: 10.6133/apjcn.2015.24.2.13.
 30. Shaw NS, Wang JL, Pan WH, Liao PC, Yang FL. Thiamin and riboflavin status of Taiwanese elementary school-children. *Asia Pac J Clin Nutr.* 2007;16:564-71. doi: 10.6133/apjcn.2007.16.s2.07.
 31. Wu SJ, Chang YH, Wei IL, Kao MD, Lin YC, Pan WH. Intake levels and major food sources of energy and nutrients in the Taiwanese elderly. *Asia Pac J Clin Nutr.* 2005;14: 211-20.
 32. Huang YC, Lee MS, Wahlqvist ML. Prediction of all-cause mortality by B group vitamin status in the elderly. *Clin Nutr.* 2012;31:191-8. doi: 10.1016/j.clnu.2011.10.010.
 33. Henriquez-Sanchez P, Sanchez-Villegas A, Doreste-Alonso J, Ortiz-Andrellucchi A, Pfrimer K, Serra-Majem L. Dietary assessment methods for micronutrient intake: a systematic review on vitamins. *Brit J Nutr.* 2009;102:S10-37. doi: 10.1017/S0007114509993126.