



Hb and dyslipidaemia as predicting markers of serum alanine aminotransferase elevation in Chinese adolescents

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Abstract

Objective: Fe is an essential element for erythropoiesis and Hb synthesis. High Hb levels affect the blood's viscosity and are associated with cardiovascular dysfunction. The aim of the present study was to examine relationships of Hb and cardiometabolic abnormalities with the risk of alanine aminotransferase (ALT) elevation in adolescents.

Design: A population-based, cross-sectional study.

Setting: National Nutrition and Health Survey in Taiwan (2010–2011, adolescents).

Subjects: Healthy adolescents aged 13–18 years.

Results: In total, 1941 adolescents (963 boys and 978 girls) were entered in the study. The mean age was 15.3 (SD 0.1) years (boys, 15.3 (SD 0.1) years; girls, 15.2 (SD 0.1) years). ALT tertile cut-off points for boys were 11 and 16 U/l, and for girls were 9 and 12 U/l. Girls without dyslipidaemia and presenting in the highest quartile (Q1) of Hb (>13.6 g/dl) were 1.89 and 3.76 times more likely to have raised serum ALT (9 and >12 U/l, respectively) than the reference (lowest quartile of Hb (Q1), <12.4 g/dl). Moreover, for those girls with dyslipidaemia, serum ALT seemed to increase with an increase in Hb levels. Specifically, girls with dyslipidaemia and Hb levels of 12.4, 13.1 and 13.6 g/dl were, respectively, 2.86, 3.53 and 5.64 times more likely to have elevated serum ALT levels (>12 U/l) than the reference (Q1 of Hb, <12.4 g/dl). The only effect found in boys was for those who had dyslipidaemia and presenting in Q4 of Hb (>15.4 g/dl), who were 7.40 times more likely to have elevated serum ALT of >16 U/l than the reference (Q1 of Hb, <14.1 g/dl).

Conclusions: Our findings suggest that an increased Hb level is a predictor of elevated serum ALT in adolescent girls with dyslipidaemia. Our study also highlights the importance of further research to establish cut-off points for Hb and its utility in diagnosing and preventing the onset of dyslipidaemia in adolescents.

Keywords

Alanine aminotransferase
Dyslipidaemia
Hb
Taiwanese adolescents
Liver function

Serum alanine aminotransferase (ALT) is a common and inexpensive laboratory assay for detecting liver diseases such as non-alcoholic fatty liver disease (NAFLD)⁽¹⁾. Serum ALT elevation is also regarded as a hepatic manifestation of the metabolic syndrome (MetS)^(2–6). Recently, a meta-analysis on the impact of serum ALT activity on MetS incidence in thirty-nine studies in adults showed a greater pooled relative risk of incident MetS for those with the

highest *v.* the lowest ALT levels⁽⁶⁾. Mechanistic links between serum ALT elevation and risks of MetS are still not fully understood. ALT is a cytosolic enzyme and is predominantly present in the liver. Elevated serum ALT levels (>40 U/l) typically reflect hepatocellular injury⁽⁷⁾. Serum ALT levels are positively correlated with BMI⁽¹⁾ and visceral fat accumulation^(8,9). When examining relationships between serum ALT activity and individual components of

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MetS in children, several authors reported a strong correlation between serum ALT activity and blood lipid profiles (e.g. TAG, LDL cholesterol and HDL cholesterol)^(4,5). The predicting effects of serum ALT levels on insulin resistance seem to be stronger in adults^(10,11) than in children^(4,5).

Dysmetabolic Fe overload syndrome, which is characterized by mild to moderate Fe overload, was first described by Deugnier *et al.* in 1992⁽¹²⁾. Dysmetabolic Fe overload syndrome is frequently associated with obesity and alterations of glucose and lipid metabolism⁽¹³⁾. It was estimated that dysmetabolic Fe overload syndrome is present in about a third of patients with both NAFLD and MetS⁽¹⁴⁾. Excessive body Fe can cause damage to the liver. For example, high Hb levels are positively correlated with serum ALT concentrations⁽¹⁵⁾ and predict the development of NAFLD⁽¹⁶⁾. The consensus view is that an obesity-related inflammatory environment⁽¹⁷⁾ may alter Fe metabolism and interfere with cardiometabolic functions⁽¹⁸⁾. Analysis of hepatic genes involved in the metabolism of fatty acids and Fe in NAFLD showed that the genes involved in fatty acid metabolism were attenuated as NAFLD progressed, whereas Fe-related metabolism increased⁽¹⁹⁾. Fe-induced oxidative stress promotes hepatic injury, leading to the release of the intracellular ALT enzyme into the circulating blood.

Fe is an essential element for erythropoiesis and Hb synthesis. High Hb levels affect the blood's viscosity, which may limit blood flow and oxygen delivery to tissues. Disorders of blood viscosity are a risk factor for cardiovascular dysfunction⁽²⁰⁾. We hypothesized that high Hb levels may interfere with cardiometabolic functions and unresolved cardiometabolic stress may lead to hepatic injury. The aim of the present study was to investigate the interactive relationships between Hb levels and cardiometabolic abnormalities in relation to risks of serum ALT elevation in a general adolescent population as part of the Nutrition and Health Survey in Taiwan (NAHSIT 2010–2011, Adolescents).

Experimental methods

Study design

The Fourth National Nutrition and Health Survey in Taiwan (NAHSIT 2010–2011) was funded by the Food and Drug Administration, Ministry of Health and Welfare in Taiwan to provide continued assessment of the health and nutrition status of the people of Taiwan. The nationwide survey was conducted using a multistage, stratified, clustered sampling technique which included a wide range of age groups across all of Taiwan⁽²¹⁾. The present study analysed data on adolescents aged 13–18 years (NAHSIT 2010–2011, Adolescents). Informed parental written consent was obtained prior to enrolment into the study. The study was approved by the Research Ethics Committee of Taipei Medical University (201210005) and Academia Sinica (EC100031).

Sample inclusion and exclusion

Information on self-reported family health histories and lifestyle factors were obtained using a standardized questionnaire. Exclusion criteria were as follows: (i) individuals with missing data for clinical biochemistry (n 1236); (ii) a self-reported health history of diabetes (n 3), thyroid disease (n 3), hepatitis (n 2), nephritis (n 3), urinary tract infection (n 34) or arthritis (n 8); and (iii) >2 drinks of alcohol/d (n 1). As such, 1941 adolescent participants (963 males and 978 females) were entered for analysis.

Data collection and laboratory measurements

Three anthropometric measures were collected in the present study. Waist circumference measurements were taken at the midpoint between the lower edge of the ribcage and the top of the iliac crest. Height and weight of participants were measured simultaneously using Detecto scales (Detecto Scales, Brooklyn, NY, USA). Two blood pressure measurements were taken 30 s apart with the arm at the level of the heart. A third measurement was taken if the second measurement differed substantially from the first (e.g. >10 mmHg). The two closest blood pressure values were averaged to obtain the mean blood pressure. Biochemical data were obtained from 8-h fasting blood samples. Heparinized whole-blood samples were collected for on-site measurement of Hb. Peripheral venous blood samples were collected in tubes containing EDTA and centrifuged at 4°C, and serum was stored at –80°C until analysis. Biochemistry analyses included total cholesterol, LDL cholesterol, HDL cholesterol, TAG, fasting blood glucose, uric acid, C-reactive protein, creatinine, ALT, aspartate aminotransferase and amylase.

Definitions of metabolic factors in the study population

The BMI was calculated as weight/height² (kg/m²). Age- and sex-specific cut-off points for BMI were used to define overweight and obesity in adolescents according to guidelines of the Department of Health, Taiwan⁽²²⁾. Hypertension was diagnosed as blood pressure values exceeding the 90th percentile for age and sex. Diabetes was defined as fasting serum glucose \geq 110 mg/dl. Dyslipidaemia was classified as adolescents with the presence of any blood lipid values exceeding the 90th percentile or below the 10th percentile: (i) TAG \geq 112 mg/dl; (ii) total cholesterol \geq 197 mg/dl; (iii) LDL cholesterol \geq 121.2 mg/dl; and (iv) HDL cholesterol < 40 mg/dl. ALT tertile (T) cut-off points for boys were 11 and 16 U/l, and for girls were 9 and 12 U/l. Hb quartiles (Q) for boys were 14.1, 14.7 and 15.4 g/dl, and for girls were 12.4, 13.1 and 13.6 g/dl.

Statistical analyses

Statistical analyses were performed using the statistical software package SAS version 9.22. Categorical data are presented as numbers and percentages, and were

assessed with a χ^2 test. Continuous data are presented as means and standard deviations, and were assessed with a two-sample *t* test. Differences between two independent samples were analysed by the Wilcoxon rank-sum test for non-parametric data. Logistic regression models were used to estimate the odds ratio of the dependent variable (serum ALT) and independent variables (age, sex, BMI, C-reactive protein, amylase, uric acid, creatine, Hb, diabetes, hypertension and dyslipidaemia) and the 95% confidence interval. In Table 2, the OR was expressed as ALT T3 *v.* ALT T1 (reference) after adjusting for ALT T2 *v.* T1. To further characterize the interactive relationships between Hb and dyslipidaemia in relation to the risk of ALT elevation, a binary logistic model was employed (Table 3). $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

In total, 1941 adolescents (963 boys and 978 girls) participated in the present study. The mean age of study participants was 15.2 (SD 1.9) years (boys, 15.3 (SD 0.1) years; girls, 15.2 (SD 0.1) years). Their mean BMI was 21.4 (SD 4.2) kg/m² (boys, 21.9 (SD 0.2) kg/m²; girls, 21.0 (SD 0.1) kg/m²; $P < 0.001$). Mean ALT was 14.8 (SD 13.3) U/l (boys, 17.7 (SD 16.3) U/l; girls, 12.1 (SD 8.7) U/l; $P < 0.001$). Mean Hb was 13.8 (SD 1.5) g/dl (boys, 14.7 (SD 1.0) g/dl; girls, 12.9 (SD 1.3) g/dl; $P < 0.001$). The prevalence of diabetes, hypertension and dyslipidaemia were 11.4% (boys, 16.0%; girls, 9.4%; $P < 0.001$), 16.7% (boys, 24.2%; girls, 9.4%; $P < 0.001$) and 19.1% (boys, 18.0%; girls, 20.2%), respectively. Table 1 shows characteristics of the study population in relation to hepatic injury (defined as serum ALT > 40 U/l). Fifty-four of 963 boys (5.6%) and twelve of 978 girls (1.2%) had elevated ALT levels. Adolescents with hepatic injury were heavier and had higher levels of AST, systolic blood pressure, Hb, total cholesterol, TAG, LDL cholesterol, uric acid, creatine and C-reactive protein, and lower HDL cholesterol levels (all $P < 0.05$; Table 1).

Associations between serum biomarkers and serum alanine aminotransferase concentrations

We next investigated the associations between serum biomarkers and risk of ALT elevation in relation to sex. After adjusting for age, factors such as BMI, C-reactive protein, uric acid, Hb, hypertension and dyslipidaemia were significantly associated with serum ALT levels (model A; Table 2). After adjusting for covariates including the T2 *v.* T1 effect, the OR were markedly higher for dyslipidaemia (OR = 1.85; 95% CI 1.16, 2.93), BMI (OR = 1.34; 95% CI 1.27, 1.41) and Hb (OR = 1.21; 95% CI 1.02, 1.42) for boys with the highest ALT (T3) compared with the lowest (T1; multivariate model: boys; Table 2). The predictive effects of dyslipidaemia (OR = 1.52; 95% CI 1.16, 16.16), Hb (OR = 1.30; 95% CI 1.47, 2.13) and BMI

Table 1 Baseline characteristics of the study population in relation to hepatic injury: healthy adolescents aged 13–18 years (*n* 1941), Fourth National Nutrition and Health Survey in Taiwan (NAHSIT 2010–2011, Adolescents)

Characteristic*	ALT ≤ 40 U/l (<i>n</i> 1875)		ALT > 40 U/l (<i>n</i> 66)		<i>P</i> value†
	Mean or %	SD or <i>n</i> / <i>N</i>	Mean or %	SD or <i>n</i> / <i>N</i>	
Male	94.4	909/963	5.6	54/963	<0.0001
Female	98.8	966/978	1.2	12/978	
AST (U/l)	18.8	4.7	41.7	19.0	<0.0001
ALT (U/l)	13.0	5.8	68.3	36.7	<0.0001
Anthropometry					
BMI (kg/m ²)	21.2	3.9	29.1	5.8	<0.0001
WC (cm)	75.7	9.6	95.4	14.8	<0.0001
WHR	0.81	0.05	0.89	0.06	0.001
Serum biochemistry					
SBP (mmHg)	103.5	10.8	113.4	12.5	<0.0001
DBP (mmHg)	59.9	7.9	63.3	9.4	0.042
TC (mg/dl)	159.9	27.7	170.3	30.8	0.003
HDL-C (mg/dl)	55.1	12.4	44.9	10.4	<0.0001
LDL-C (mg/dl)	90.5	24.1	104.6	28.7	0.001
TAG (mg/dl)	71.3	31.5	104.6	45.4	<0.0001
Fasting glucose (mg/dl)	94.8	7.9	96.7	9.0	0.067
Hb (g/dl)	13.8	1.5	14.6	1.3	<0.0001
Amylase (mg/dl)	66.3	21.0	56.9	16.6	<0.0001
UA (mg/dl)	5.7	1.4	7.2	1.7	<0.0001
CRP (ng/ml)	0.15	0.24	0.21	0.23	0.044
CREA (mg/dl)	0.66	0.16	0.70	0.17	0.038

AST, aspartate aminotransferase; ALT, alanine aminotransferase; WC, waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; UA, uric acid; CRP, C-reactive protein; CREA, creatine.

*Categorical data (sex) are presented as percentage and number of observations/number of participants; continuous data are presented as mean and standard deviation.

†Differences between groups were analysed by a two-sample *t* test.

(OR = 1.20; 95% CI 1.01, 1.35) for raised ALT levels in girls were similar to those of boys (multivariate model: girls; Table 2).

Interactive relationships of Hb and dyslipidaemia with the risk of serum alanine aminotransferase elevation

To further classify the interactive relationships of Hb and dyslipidaemia with the risk of ALT elevation, a categorical logistic model was employed. Adjusted OR for the risk of serum ALT elevation in relation to the Hb level and dyslipidaemia are shown in Table 3. Girls without dyslipidaemia and presenting in the highest quartile (Q4) of Hb (>13.6 g/dl) were 1.89 and 3.76 times more likely to have raised serum ALT (9 and >12 U/l, respectively) than the reference (lowest quartile (Q1) of Hb, <12.4 g/dl). Moreover, for those girls with dyslipidaemia, serum ALT seemed to increase with an increase in Hb levels. Specifically, girls with dyslipidaemia and Hb levels of 12.4, 13.1 and 13.6 g/dl were, respectively, 2.86, 3.53 and 5.64 times more likely to have elevated serum ALT levels (>12 U/l) than the reference (Q1 of Hb, <12.4 g/dl). The only effect

Table 2 Comparisons of odds ratios and 95 % confidence intervals for risk factors associated with serum alanine aminotransferase elevation among healthy adolescents aged 13–18 years (*n* 1941), Fourth National Nutrition and Health Survey in Taiwan (NAHSIT 2010–2011, Adolescents)

	Model A*			Multivariate model†		
	OR‡	95 % CI	<i>P</i> value	OR‡	95 % CI	<i>P</i> value
Boys§						
BMI (kg/m ²)	1.27	1.23, 1.32	<0.0001	1.34	1.27, 1.41	<0.0001
CRP (ng/ml)	1.84	1.02, 3.31	0.042	1.85	0.91, 3.78	0.091
Amylase (mg/dl)	1.00	0.99, 1.00	0.163			
UA (mg/dl)	1.43	1.30, 1.58	<0.0001	1.03	0.90, 1.18	0.675
CREA (mg/dl)	2.25	0.83, 6.11	0.111			
Hb (g/dl)	1.24	1.09, 1.40	0.001	1.21	1.02, 1.42	0.026
Diabetes	1.50	0.80, 2.83	0.210			
Hypertension¶	2.34	1.70, 3.22	<0.0001	1.11	0.70, 1.76	0.659
Dyslipidaemia**	3.42	2.46, 4.76	<0.0001	1.85	1.16, 2.93	0.010
Girls§						
BMI (kg/m ²)	1.14	1.10, 1.19	0.446	1.20	1.01, 1.35	0.049
CRP (ng/ml)	1.71	0.94, 3.14	0.080			
Amylase (mg/dl)	1.00	1.00, 1.01	0.446			
UA (mg/dl)	1.48	1.28, 1.72	<0.0001	0.89	0.23, 3.41	0.865
CREA (mg/dl)	1.71	0.41, 7.17	0.461			
Hb (g/dl)	1.33	1.16, 1.51	<0.0001	1.30	1.47, 2.13	0.003
Diabetes	1.08	0.31, 3.76	0.900			
Hypertension¶	1.41	1.00, 1.97	0.048	0.86	0.04, 16.57	0.918
Dyslipidaemia**	1.70	1.23, 2.34	0.001	1.52	1.16, 16.16	0.010

CRP, C-reactive protein; UA, uric acid; CREA, creatine; ALT, alanine aminotransferase; T, tertile.

Significant OR are indicated in bold font.

*Model A is adjusted for age.

†Multivariate model is adjusted for age, BMI, inflammatory markers (CRP, amylase, UA and CREA), Hb, diabetes, hypertension and dyslipidaemia.

‡The OR was expressed as ALT T3 v. ALT T1 (reference) after adjusting for ALT T2 v. T1.

§Serum ALT T by sex: males, 11 and 16 U/l; females, 9 and 12 U/l.

||Diabetes: fasting serum glucose \geq 110 mg/dl.

¶|Hypertension: values exceeding the 90th percentile for age and sex.

**Dyslipidaemia defined as presence of any of the following criteria: (i) TAG \geq 112 mg/dl; (ii) total cholesterol \geq 197 mg/dl; (iii) LDL cholesterol \geq 121.2 mg/dl; or (iv) HDL cholesterol $<$ 40 mg/dl.

Table 3 Adjusted odds ratios and 95 % confidence intervals for risk of serum alanine aminotransferase elevation in relation to dyslipidaemia and Hb levels among healthy adolescents aged 13–18 years (*n* 1941), Fourth National Nutrition and Health Survey in Taiwan (NAHSIT 2010–2011, Adolescents)

	Hb†	ALT T2 v. T1*			ALT T3 v. T1*		
		OR‡	95 % CI	<i>P</i> value	OR‡	95 % CI	<i>P</i> value
Boys							
DL(–) (<i>n</i> 730)	Q1	Ref.	–	–	Ref.	–	–
	Q2	0.83	0.49, 1.40	0.486	1.09	0.58, 2.06	0.787
	Q3	0.85	0.50, 1.43	0.533	1.06	0.56, 1.98	0.861
	Q4	1.06	0.56, 1.98	0.861	1.45	0.78, 2.72	0.242
DL(+) (<i>n</i> 233)	Q1	0.89	0.20, 4.01	0.882	3.51	0.88, 14.04	0.076
	Q2	1.29	0.37, 4.56	0.692	2.42	0.66, 8.92	0.184
	Q3	0.74	0.28, 1.97	0.548	1.51	0.55, 4.20	0.426
	Q4	3.86	0.83, 17.92	0.085	7.40	1.57, 35.01	0.012
Girls							
DL(–) (<i>n</i> 886)	Q1	Ref.	–	–	Ref.	–	–
	Q2	1.12	0.68, 1.83	0.662	1.44	0.83, 2.50	0.197
	Q3	1.65,	0.96, 2.85	0.073	1.79	0.97, 3.33	0.065
	Q4	1.89	1.05, 3.39	0.033	3.76	2.00, 7.04	<0.0001
DL(+) (<i>n</i> 92)	Q1	1.05	0.36, 3.10	0.929	1.01	0.31, 3.30	0.991
	Q2	2.03	0.75, 5.48	0.163	2.86	1.01, 8.07	0.048
	Q3	2.21	0.82, 5.39	0.116	3.53	1.26, 9.85	0.016
	Q4	2.56	0.96, 6.83	0.061	5.64	2.08, 15.32	0.001

ALT, alanine aminotransferase; T, tertile; DL(–), without dyslipidaemia; DL(+), with dyslipidaemia; Q, quartile; Ref., reference category.

Significant OR are indicated in bold font.

*Serum ALT T by sex: males, 11 and 16 U/l; females, 9 and 12 U/l.

†Hb quartile by sex: males, 14.1, 14.7 and 15.4 g/dl; females, 12.4, 13.1 and 13.6 g/dl.

‡OR adjusted for age and BMI.



found in boys was for those who had dyslipidaemia and presenting in Q4 of Hb (>15.4 g/dl), who were 7.40 times more likely to have elevated serum ALT of >16 U/l than the reference (Q1 of Hb, <14.1 g/dl).

Discussion

Our study found that dyslipidaemia and high Hb levels were associated with an increased risk of high ALT levels in ethnic Chinese (i.e. Taiwanese) adolescents. The hypothesized mechanisms by which Hb–lipid interactions may be associated with abnormal liver function include advanced glycation end products (AGE) and their receptors (RAGE)^(23,24); and hepcidin levels⁽²⁵⁾. Obese individuals tend to replace carbohydrates with animal protein and fat as sources of energy⁽²⁶⁾. Meat, particularly dry-heated red meat, is a rich source of haem Fe, fat and AGE⁽²⁷⁾. Fu and co-workers showed that N^{ϵ} -(carboxymethyl)lysine is formed during Cu-catalysed oxidation of PUFA in the presence of protein⁽²⁸⁾. Estevez *et al.* reported that myoglobin is a good predictive marker for protein carbonyl formation⁽²⁷⁾. The interaction of AGE and RAGE plays a key role in the development and progression of NAFLD^(29,30). Lastly, the hepcidin level, a key regulator of Fe metabolism, may play an important role in Hb levels and hepatic function. By investigating thirty-six human liver specimens derived from liver carcinoma or transplantation for cirrhosis, Detivaud *et al.* found a positive relationship between liver hepcidin mRNA and Hb levels and a negative relationship between hepatic fibrosis status and hepcidin mRNA levels⁽²⁵⁾.

Girls appear to be more sensitive to Hb–lipid interactions than boys with regard to serum ALT elevation. The reason for this sex difference is unclear. However, it is interesting to speculate that this sex difference may be due to the interactive relationship between oestrogen and erythrocytes. Reproductive-aged women have lower mean Hb levels (12%) than men. A recent study by Hou *et al.* showed that oestrogen regulates Fe homeostasis via down-regulating hepatic hepcidin expression via an oestrogen response element⁽³¹⁾. When hepcidin concentrations are low, Fe enters the blood plasma at a higher rate resulting in high body Fe levels. Although oestrogen seems to have beneficial effects on Fe levels, it was also associated with deleterious changes in membrane lipids of erythrocytes. For example, a study by Cho and co-workers demonstrated that oestrogen-induced dyslipidaemia resulted in changes in the fatty acid composition of membrane lipids of erythrocytes, which in turn increased osmotic fragility⁽³²⁾. Le Petit-Thevenin *et al.* showed that oestrogen increased erythrocyte susceptibility to peroxidation generated by incubation with hydrogen peroxide⁽³³⁾. Decreased erythrocyte membrane fluidity and altered lipid compositions were associated with human liver diseases⁽³⁴⁾.

Our study suggests that Hb testing in adolescents with dyslipidaemia may identify those with serum ALT

elevation. Jiang and colleagues also proposed that Fe biomarkers (Hb and ferritin) combined with TAG could serve as an indicator for a diagnosis of NAFLD in Chinese adults⁽³⁵⁾. Although a liver biopsy remains the gold standard for diagnosing NAFLD, a histological diagnosis is often not possible in community-based studies. In Taiwan, a diagnosis of NAFLD is largely based on ultrasonography and serum ALT due to the asymptomatic nature of the disease and invasiveness and discomfort associated with a liver biopsy. However, the use of ultrasonography is limited to diagnosing advanced or fully developed NAFLD. Furthermore, ultrasonography cannot identify the presence of non-alcoholic steatohepatitis or reliably distinguish changes in fibrosis from non-alcoholic steatohepatitis. Our study suggests that Hb can serve as an additional biomarker for detecting liver disease. However, there is no consensus on the ‘normal reference range’ for Hb levels in adults and adolescents. The absence of relevant cut-off points for Hb limits its clinical utility.

Several limitations of the present study should be taken into account when interpreting the results. First, the number of adolescents with abnormal ALT (>40 U/l) levels was relatively small. Second, the liver function diagnosis was based on the serum ALT threshold and not on ultrasonography due to budget constraints. Third, exclusion of hepatitis viral infections was based on a self-reported health history and not on hepatitis surface antigens. Previously, the hepatitis B virus was endemic in Taiwan, but the infection rate decreased in adolescents and young adults after the launch of the world’s first universal vaccination programme in 1984, which involved vaccinating newborns of infectious mothers and was later expanded to all newborns in 1986. A recent report investigated 8733 senior-high-school students and reported overall hepatitis B surface antigen (HBsAg) and anti-HBs-positive rates of 1.9% and 48.3%, respectively⁽³⁶⁾. Lastly, our study did not measure the pubertal stage, and therefore we cannot discriminate the potential influence of sexual hormones on Hb concentrations.

Conclusions

Our findings suggest that an increased Hb level is a predictor of elevated serum ALT in adolescent girls with dyslipidaemia. Our study also highlights the importance of new studies aiming to establish cut-off points for Hb levels and its utility in diagnosing and preventing the onset of cardiometabolic abnormalities particularly in female adolescents.

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References

1. Welsh JA, Karpen S & Vos MB (2013) Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988–1994 to 2007–2010. *J Pediatr* **162**, 496–500.
2. Park JH, Kim SH, Park S *et al.* (2014) Alanine aminotransferase and metabolic syndrome in adolescents: the Korean National Health and Nutrition Examination Survey Study. *Pediatr Obes* **9**, 411–418.
3. Fu CC, Chen MC, Li YM *et al.* (2009) The risk factors for ultrasound-diagnosed non-alcoholic fatty liver disease among adolescents. *Ann Acad Med Singapore* **38**, 15–17.
4. Di Bonito P, Sanguigno E, Di Fraia T *et al.* (2009) Association of elevated serum alanine aminotransferase with metabolic factors in obese children: sex-related analysis. *Metabolism* **58**, 368–372.
5. Park HS, Han JH, Choi KM *et al.* (2005) Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. *Am J Clin Nutr* **82**, 1046–1051.
6. Liu Z, Que S, Ning H *et al.* (2013) Elevated alanine aminotransferase is strongly associated with incident metabolic syndrome: a meta-analysis of prospective studies. *PLoS One* **8**, e80596.
7. Adams LA, Angulo P & Lindor KD (2005) Nonalcoholic fatty liver disease. *CMAJ* **172**, 899–905.
8. Song HR, Yun KE & Park HS (2008) Relation between alanine aminotransferase concentrations and visceral fat accumulation among nondiabetic overweight Korean women. *Am J Clin Nutr* **88**, 16–21.
9. Ayonrinde OT, Olynyk JK, Beilin LJ *et al.* (2011) Gender-specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. *Hepatology* **53**, 800–809.
10. Kunutsor SK, Apekey TA & Walley J (2013) Liver aminotransferases and risk of incident type 2 diabetes: a systematic review and meta-analysis. *Am J Epidemiol* **178**, 159–171.
11. Wang CS, Chang TT, Yao WJ *et al.* (2012) Impact of increasing alanine aminotransferase levels within normal range on incident diabetes. *J Formos Med Assoc* **111**, 201–208.
12. Deugnier YM, Loreal O, Turlin B *et al.* (1992) Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their biochemical correlations. *Gastroenterology* **102**, 2050–2059.
13. Riva A, Trombini P, Mariani R *et al.* (2008) Reevaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. *World J Gastroenterol* **14**, 4745–4752.
14. Dongiovanni P, Fracanzani AL, Fargion S *et al.* (2011) Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* **55**, 920–932.
15. Bai CH, Owaga E, Cheng SY *et al.* (2014) Relationship between hemoglobin levels and risk for suspected non-alcoholic fatty liver in Taiwanese adults. *Chin J Physiol* **57**, 286–294.
16. Yu C, Xu C, Xu L *et al.* (2012) Serum proteomic analysis revealed diagnostic value of hemoglobin for nonalcoholic fatty liver disease. *J Hepatol* **56**, 241–247.
17. Chang JS, Li YL, Lu CH *et al.* (2014) Interleukin-10 as a potential regulator of hepcidin homeostasis in overweight and obese children: a cross-sectional study in Taiwan. *Nutrition* **30**, 1165–1170.
18. Yilmaz Y, Senates E, Ayyildiz T *et al.* (2012) Characterization of nonalcoholic fatty liver disease unrelated to the metabolic syndrome. *Eur J Clin Invest* **42**, 411–418.
19. Mitsuyoshi H, Yasui K, Harano Y *et al.* (2009) Analysis of hepatic genes involved in the metabolism of fatty acids and iron in nonalcoholic fatty liver disease. *Hepatol Res* **39**, 366–373.
20. Metivier F, Marchais SJ, Guerin AP *et al.* (2000) Pathophysiology of anaemia: focus on the heart and blood vessels. *Nephrol Dial Transplant* **15**, Suppl. 3, 14–18.
21. Pan WH, Lee MS, Chuang SY *et al.* (2008) Obesity pandemic, correlated factors and guidelines to define, screen and manage obesity in Taiwan. *Obes Rev* **9**, Suppl. 1, 22–31.
22. Tan CE, Ma S, Wai D *et al.* (2004) Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? *Diabetes Care* **27**, 1182–1186.
23. Kimura Y, Hyogo H, Yamagishi S *et al.* (2010) Atorvastatin decreases serum levels of advanced glycation endproducts (AGEs) in nonalcoholic steatohepatitis (NASH) patients with dyslipidemia: clinical usefulness of AGEs as a biomarker for the attenuation of NASH. *J Gastroenterol* **45**, 750–757.
24. Moy KA, Jiao L, Freedman ND *et al.* (2013) Soluble receptor for advanced glycation end products and risk of liver cancer. *Hepatology* **57**, 2338–2345.
25. Detivaud L, Nemeth E, Boudjema K *et al.* (2005) Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. *Blood* **106**, 746–748.
26. Chang JS, Chen YC, Owaga E *et al.* (2014) Interactive effects of dietary fat/carbohydrate ratio and body mass index on iron deficiency anemia among Taiwanese women. *Nutrients* **6**, 3929–3941.
27. Estevez M (2011) Protein carbonyls in meat systems: a review. *Meat Sci* **89**, 259–279.
28. Fu MX, Requena JR, Jenkins AJ *et al.* (1996) The advanced glycation end product, N^ε-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. *J Biol Chem* **271**, 9982–9986.
29. Christman AL, Lazo M, Clark JM *et al.* (2011) Low glycated hemoglobin and liver disease in the US population. *Diabetes Care* **34**, 2548–2550.
30. Basta G, Navarra T, De Simone P *et al.* (2011) What is the role of the receptor for advanced glycation end products–ligand axis in liver injury? *Liver Transpl* **17**, 633–640.



31. Hou Y, Zhang S, Wang L *et al.* (2012) Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. *Gene* **511**, 398–403.
32. Cho BH, Smith TL, Park JR *et al.* (1988) Effects of estrogen-induced hyperlipidemia on the erythrocyte membrane in chicks. *Lipids* **23**, 853–856.
33. Le Petit-Thevenin J, Lericque B, Nobili O *et al.* (1991) Estrogen modulates phospholipid acylation in red blood cells: relationship to cell aging. *Am J Physiol* **261**, C423–C427.
34. Owen JS, Bruckdorfer KR, Day RC *et al.* (1982) Decreased erythrocyte membrane fluidity and altered lipid composition in human liver disease. *J Lipid Res* **23**, 124–132.
35. Jiang Y, Zeng J & Chen B (2014) Hemoglobin combined with triglyceride and ferritin in predicting non-alcoholic fatty liver. *J Gastroenterol Hepatol* **29**, 1508–1514.
36. Wu TW, Lin HH & Wang LY (2013) Chronic hepatitis B infection in adolescents who received primary infantile vaccination. *Hepatology* **57**, 37–45.