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The longitudinal increments of serum alanine aminotransferase increased the incidence risk of metabolic syndrome: A large cohort population in China

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ABSTRACT

Background: Although alanine aminotransferase (ALT) is well known to be associated with metabolic syndrome (MetS), prospective data on longitudinal increments in ALT activities and incident cases of MetS are limited. We analyzed the impact of longitudinal increments of ALT on MetS based on a health check-up population in China.
Methods: A total of 4491 subjects free of MetS who completed at least two annual health examinations during March 2010 to April 2016 were enrolled in this cohort study. The MetS was defined according to the Joint Interim Statement criteria 2009. The RRs of incident MetS were estimated by using the Cox model and the Joint model in R software.
Results: The cumulative incidence of MetS was 18.55% during the 7 years of follow-up. In the Cox model, the estimated RR of developing MetS was 1.751 (95% CI = 1.532–2.000) for 1 unit augmented in *LNALT*-0 level. In the Joint model, the estimated RR of developing MetS was 3.626 (95% CI = 2.721–4.831) for 1 unit augmented in *LNALT* activity longitudinally.
Conclusions: The longitudinal increment of individuals' ALT activity over time increased the incidence risk of MetS and the effects generated by longitudinal increments of ALT on MetS was higher than that generated by baseline ALT.

1. Introduction

Metabolic syndrome (MetS) is a cluster of disorders, including central obesity, dyslipidemia, impaired glucose tolerance, and hypertension [1]. Some previous studies have shown that the main clinical outcomes of MetS are cardiovascular disease (CVD) and Type 2 diabetes mellitus (T2DM) [2–4]. These two diseases are major threats to human health and quality of life [5]. Therefore, early detection of high-risk MetS in individuals becomes an important issue. A study indicated that patients with MetS usually manifested as abnormal accumulation of triglyceride (TG) in the liver and hepatic insulin resistance [6]. Kogiso T et al. showed that fatty liver could be a part of or, at least, a predictor of MetS [7]. Serum alanine aminotransferase (ALT) activities rise as a consequence of hepatocellular injury (including liver fat), and this can effectively identify ongoing liver disease. The prevalence of elevated ALT is higher in individuals with MetS [8]. Several studies have demonstrated that elevated serum ALT activities have a positive association with MetS-related disease such as T2DM [9] and CVD [10]. Elevated ALT activities, even within the usual reference range (≤ 40 U/l), were confirmed to be significantly correlated to MetS [11,12].

At present, a wide range of cross-sectional and prospective studies have investigated the relationship between baseline ALT and MetS [13,14]. Nevertheless, these previous studies, and even other cohort-studies [15–17], mainly used the traditional statistical analysis methods (for instance, the general Cox model) to analyze the effects of baseline ALT on MetS. To our knowledge, no one evaluated the effect of the longitudinal increments in ALT activities over time on the incidence risk of MetS using the Joint Model.

To clarify the association between longitudinal increments of ALT and the incidence risk of MetS, our study performed a dynamic health

Abbreviations: MetS, metabolic syndrome; FPG, fasting plasma glucose; IR, insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; MetS, metabolic syndrome; ALT, alanine aminotransferase; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; FPG, fasting plasma glucose; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; IR, insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure

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examination cohort study, which included repeated observations on the same subjects. At the same time, cohort data was analyzed using the Joint Model which fully considered the longitudinal increments in ALT, the association of repeated measurements and the random effects among individuals. These are considerations the traditional analysis could not have achieved.

2. Material and methods

2.1. Study population

In this longitudinal study, 10,858 subjects who underwent the health check-up in the Second Hospital affiliated to Dalian Medical University of China during March 2010 to April 2016 were included. Data of each subject's initial health examination was defined as baseline data and the time of occurring MetS was defined as the terminal follow-up. A total of 6367 subjects were excluded based on records for the following reasons: 1) subjects who had already been diagnosed at baseline with MetS were excluded (n = 2409); 2) subjects with a history of hypertension, diabetes, CVD, coronary heart disease, viral hepatitis, liver cirrhosis and autoimmune liver disease (n = 1676); 3) subjects with baseline missing data of ALT and MetS components such as, height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), TG, high density lipoprotein cholesterol (HDL−C) (n = 701). There remained 6072 eligible subjects who could be involved in the cohort study. However, 1581 of the 6072 subjects did not attend any follow-up visit. These subjects have completed the examination of MetS components data and ALT measurements only once were excluded from the study during the follow-up period. In total 4491 subjects were included in our study (Fig. 1). The study was approved by the institutional ethics committee of Dalian Medical University.

2.2. Anthropometric and laboratory measurements

The subjects were advised against consuming alcoholic drinks and coffee before their health examinations. Body height and weight were measured to the nearest 1 cm and 1 kg, respectively, while the subjects wore lightweight clothes and stood upright without shoes. Blood pressure was measured using an electronic sphygmomanometer in a sitting position after the subjects had been at rest for at least 5 min. The first measurement value in the normal reference range was recorded as the subject's blood pressure value. If it was in the abnormal range it was re-measured after the subject was at rest for 20 min. The minimum value of the 2 measurements was taken to be the final blood pressure value.

Blood samples were collected from elbow veins in the morning after the subjects had fasted for 12 h overnight and detected blood samples in 10 to 30 min after the collection. The serum concentrations of FPG, TG, HDL-C and ALT were measured on a Siemens ADVIA 2400 automatic biochemical analyzer. FPG was determined by means of hexokinase method with an intra-assay CV of < 1.0% and an inter-assay CV of < 2.0%. TG was determined by the phosphoglyceride oxidase-peroxidase method with an intra-assay CV of < 0.9% and an inter-assay CV of < 1.5%. HDL-C was determined by means using enzyme selective protection method with an intra-assay CV of < 2.0% and an inter-assay CV of < 3.0%. ALT was determined by means of the velocity method with an intra-assay CV of < 3.2% and an inter-assay CV of < 4.0%.

2.3. Definition of metabolic syndrome

We used the Joint Interim Statement criteria of 2009 [18] for making MetS diagnoses. A subject was claimed as having MetS if he or she had three or more of the following risk factors: (1) obesity: BMI ≥ 25 kg/m^2; (2) elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator): SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg; (3) increaseded TG (drug treatment for increased TG is an alternate indicator): ≥ 150 mg/dl (1.7 mmol/l); (4) Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator): < 40 mg/dl (1.0 mmol/l) in males, < 50 mg/dl (1.3 mmol/l) in females and (5) Elevated FPG (drug treatment of elevated glucose is an alternate indicator): ≥ 100 mg/dl. The definition required waist circumference (WC) to be taken to diagnose obesity. In China, the cutoff points of WC were ≥ 85 cm in men and ≥ 80 cm in women [19]. However, in our study, WC was not measured because of the limited health check-up site. BMI was taken as a substitute for the component of obesity, which is strongly correlated with WC in patients with MetS [20,21].

2.4. Explanation for variables

A list of variables used in the model with an explanation for each is shown in Table 1. The dependent variable of ALT was converted to the *LNALT* so as to satisfy the requirement of symmetric distribution of the Joint Model. In order to explain the implication of the intercept in the model, the subject's baseline age was centralized by subtracting the median of the baseline ages for all subjects.

2.5. Comparison of annual average changes in ALT

The annual average change in the MetS group (MetS occurred during follow-up) and non-MetS group (MetS not occurred during follow-up) of ALT was compared by gender stratification.

$$k = (y_{the\ last\ follow-up\ time} - y_{the\ first\ follow-up\ time})/years\ of\ follow-up$$

In the formula, y stands for the measured value of ALT and k stands for the average annual change in the given liver marker.

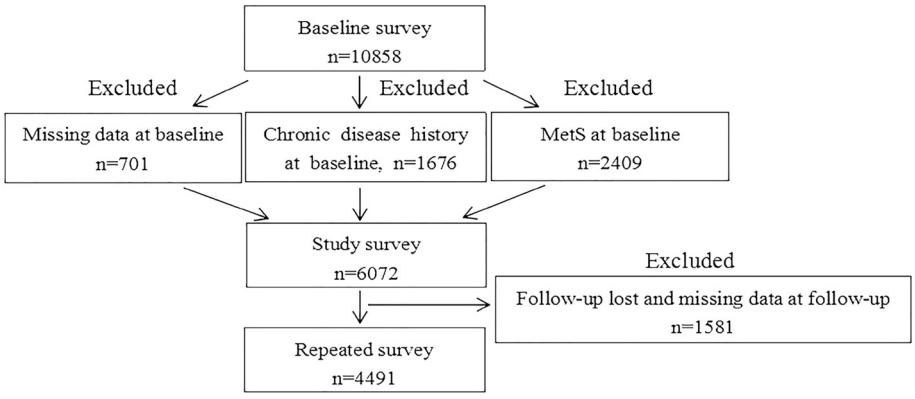

Fig. 1. Frame of the study design and numbers of subjects.

Table 1
Covariates used in analyses of Cox model and Joint model.

Variables	Explanation
Time	the interval between each health examination date and the first health examination date, the unit was years
Years	the interval between the last health examination date and the first health examination date, the unit was years
Age	the baseline age after centralized, the unit was years old
Gender	1 if male, 0 if female
State	1 if MetS, 0 if no MetS
LNALT	the ALT level after natural logarithmic conversion
LNALT-0	the baseline ALT level after natural logarithmic conversion

2.6. The general Cox model

In this study, we performed a general Cox model by introducing time-independent covariates, such as *LNALT-0, age* and *gender*. Then, according to the principle of backward stepwise regression, non-significant covariates were excluded. The final model only contained the significant ($P < .05$) covariates.

2.7. The Joint model

A Joint Model [22–24] typically combines a Linear Mixed-Effects Model (LME) for longitudinal measurement data and a Cox Model for the time-to-event via shared parameters, which can assess the impact of time-varying measurements on the survival outcomes. The Joint Model is specified as:

$$\{h_i\{t|M_i(t),x_i\} = h_0(t)\exp\{\Sigma\beta_i x_i + \alpha m_i(t)\}^{y_{ij} = m_i(t) + \varepsilon_{ij} = b_{0i} + b_{1i}t_{ij} + \varepsilon_{ij} = b_{00} + u_{0i} + (b_{10} + u_{1i})t_{ij} + \varepsilon_{ij}}$$

The y_{ij} denotes the observed value of a specific variable for the i^{th} (i = 1,2, …n) subject at the j^{th} (j=0,1…,t) follow-up time point. The $m_i(t)$ denotes the true value of y_{ij}. In the longitudinal sub-model, b_{0i} and b_{1i} represent the intercept and slope for the i^{th} subject. The (b_{00}, b_{10}) and (u_{0i}, u_{1i}) are the fixed and the random effects for the intercept and the slope term, respectively. The ε_{ij} is the random error for the i^{th} subject at the j^{th} follow-up time points with $(\varepsilon_{ij}|\sigma) \sim N(0, \sigma^2)$. Also, both random effects (u_{0i},u_{1i}) are described by σ_{u0},σ_{u1}.

The $h_i(t)$ denotes the hazard function for the i^{th} subject with covariates, $M_i(t)$ and x_i, at time t. The baseline hazard is given by $h_0(t)$. $M_i(t)$ is the whole longitudinal history of the marker levels up to time t. The x_i represents a certain time-independent covariate of $h_i(t)$. The β_i denotes the regression coefficient of x_i and α stands for the association parameter assessing the relationship between the longitudinal measurement and the survival sub-model.

In this study, we first performed a LME sub-model with a random intercept and a random slope of time for LNALT. The independent covariates, *time, age, gender, time:age, time:gender, and age:gender* were all entered into the model. Then, based on the principle of backward stepwise regression, each covariate was tested one by one, and non-significant covariates were excluded from the model. The final model, only containing the significant ($P < .05$) covariates, was determined by the Akaike Information Criterion (AIC). Second, we fitted a Cox sub-model of the Joint Model by introducing time-independent covariates, such as *LNALT-0, age and gender*. Then fitting the LME sub-model and the Cox sub-model via the association parameter α with the JM package in R software. Alike, the final Joint Model met on the minimum AIC and only contained statistically significant ($P < .05$) covariates.

2.8. Statistical approach

Data is expressed as the median and quartile range for non-normally distributed variables and compared by Wilcoxon rank sum test. Categorical data was described by proportion and compared using the Chi-square test. The basic descriptive analysis was performed by SPSS17.0 software and measurements of $P < .05$ were considered significant. The general Cox Model and the Joint Model were conducted using the R 3.2.3 package JM [25] to account for baseline and longitudinal correlations between *LNALT* and MetS.

3. Results

3.1. Basic characteristics

A total of 4491 subjects (1497 male and 2994 female, median baseline age: 37 years, range: 19 to 80 years) who did not have MetS at their baseline visit were enrolled in this cohort study. The baseline characteristics, such as age, gender, SBP, DBP, BMI, FPG, TG, HDL-C and ALT were all not statistically significant ($P > 0.05$) between the repeated survey and the study survey, so our repeated survey subjects are representative of the study survey (the result is shown in Table 1 of Supplementary file 1). Of these, in the repeated survey it was

Table 2
The comparison of baseline characteristics between MetS group and non-MetS group.

Variables	Male		Z	P	Female		Z	P
	MetS	non-MetS			MetS	non-MetS		
Age (y)	42.0 (32.0,50.0)	41.0 (32.0,49.0)	1.6	NS	38.0 (28.0,47.2)	34.0 (27.0,42.0)	5.2	0.000
SBP (mm Hg)	124.0 (117.0,131.0)	122.0 (113.0,130.0)	3.3	0.001	128.0 (112.0,128.0)	73.0 (66.0,80.0)	7.5	0.000
DBP (mm Hg)	76.0 (71.0,82.0)	74.0 (68.0,81.0)	4.2	0.000	73.0 (66.0,80.0)	70.0 (64.0,76.0)	5.9	0.000
BMI (kg/m^2)	24.9 (23.6,26.5)	23.1 (21.3,24.6)	13.3	0.000	23.4 (21.3,25.3)	21.4 (19.9,23.0)	12.2	0.000
FPG (mmol/l)	5.5 (5.3,5.7)	5.4 (5.1,5.7)	4.6	0.000	5.3 (5.0,5.6)	5.2 (4.9,5.4)	5.9	0.000
TG (mmol/l)	1.2 (1.0,1.6)	1.0 (0.8,1.3)	8.5	0.000	1.0 (0.8,1.3)	0.8 (0.6,1.0)	11.5	0.000
HDL-C (mmol/l)	1.1 (1.0,1.3)	1.3 (1.1,1.5)	8.1	0.000	1.3 (1.1,1.5)	1.5 (1.3,1.7)	10.3	0.000
ALT (U/l)	25.0 (19.0,35.0)	21.0 (16.0,29.0)	6.5	0.000	18.0 (14.0,25.0)	15.0 (12.0,19.0)	8.4	0.000

SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting blood glucose; TG, triglyceride; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; ALT, alanine aminotransferase.
Data are median (P_{25}, P_{75}).

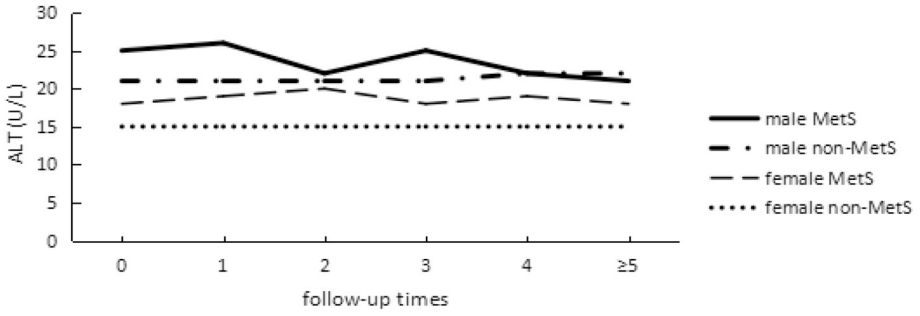

Fig. 2. Changes in the trend of average level of ALT during follow-up in the MetS group and the non-MetS group.

discovered that 833 subjects (18.55% of the total group of subjects) developed MetS in the time following the original survey. The median follow-up time was 2.0 (P_{25}, P_{75} 1.0, 4.0) years. Table 2 shows the baseline characteristics of subjects in the final repeated survey. As we can see, the baseline level of SBP, DBP, BMI, FPG, TG and ALT in the MetS group were higher than that in non-MetS group (except HDL−C), regardless of their gender.

3.2. Average activities of ALT during the follow-up in MetS group and non-MetS group

As we can see from Fig. 2, the average activities of ALT in the non-MetS group remained more or less flat during the follow-up period but the MetS group exhibited greater fluctuations.

3.3. Comparisons of the annual average rates of change in ALT activities between MeS group and non-MetS group

Table 3 presents that the annual average rates of change in ALT activities in the MetS group was significantly higher than in the non-MetS group, regardless of its gender stratification.

3.4. Association between incident MetS and LNALT at baseline

Table 4 presents the estimated parameters and RRs from the Cox Model using baseline *LNALT* (*LNALT-0*) as a covariate for prediction of MetS. As we can see, the covariates, *age, gender* and *LNALT-0* remained in the Cox Model by means of the backward likelihood regression method, which had a significant impact on the incidence of MetS. The increased RR for developing MetS was 1.017 (95% CI = 1.012–1.023) for every 1 year added to baseline *age* for one subject. The RR for *gender* predicting MetS was 2.365 (95% CI = 2.040–2.741), which indicated that males have a 2.365-fold higher risk of MetS than females. The increased RR for developing MetS was 1.751 (95% CI =1.532–2.000) for every 1 unit added to *LNALT-0* level for one subject.

Table 3
Comparison of the annual average rate of change in ALT levels between MeS group and non-MetS group.

Variables	Gender	Non-MetS	MetS	*Z*	*P*[a]
ALT	male	−0.099 (−2.875,1.995)	1.409 (−2.212,7.503)	5.953	0.000
	female	0.000 (−1.562,1.633)	1.020 (−2.003,5.176)	4.902	0.000

ALT, alanine aminotransferase.
Data are Median (P_{25}, P_{75}).
[a] *P*-value = no metabolic syndrome vs. metabolic syndrome in male or in female.

Table 4
Estimated parameter RRs from Cox model for prediction of MetS using LNALT-0 as one of covariates.

Variable	B	SE	Z	*P*	RR	RR95%CI
Age	0.017	0.003	5.891	< 2E-16 ***	1.017	1.012, 1.023
Gender	0.861	0.075	11.421	3.83E-09 ***	2.365	2.040, 2.741
LNALT-0	0.560	0.068	8.232	2.22E-16 ***	1.751	1.532, 2.000

ALT, alanine aminotransferase; RR, relative risk.

Table 5
Results of the Joint model for the association between longitudinal LNALT values and MetS.

Variable	B	SE	Z	*P*	RR	RR95%CI
Longitudinal sub-model fixed effects						
Intercept	2.775	0.008	351.911	< 0.0001		
Time	0.008	0.004	2.053	0.0401		
Age	0.010	0.001	14.555	< 0.0001		
Gender	0.403	0.014	28.075	< 0.0001		
Age:gender	−0.014	0.001	12.646	< 0.0001		
Time:gender	−0.025	0.008	3.311	0.0009		
Random effects						
$\sigma_{\delta 00}$	0.305					
$\sigma_{\delta 10}$	0.046					
ε	0.338					
Survival sub-model						
Age	0.016	0.003	5.279	< 0.0001	1.016	(1.010,1.022)
Gender	0.680	0.085	7.993	< 0.0001	1.974	(1.671,2.332)
α	1.288	0.147	8.794	< 0.0001	3.626	(2.721,4.831)

RR, relative risk.

3.5. The Joint Model result of LNALT

By the method mentioned previously, the Joint Model to investigate the effect of dynamic change in *LNALT* activities on the hazard of MetS was constructed. The result is shown in Table 5.

Table 6
Estimated RRs of MetS for different longitudinal increase of ALT at specific ALT baseline levels, under the Joint model.

Increment of ALT	ALT baseline level (U/l)			
	5	10	30	40
3 units	1.832	1.402	1.131	1.098
5 units	2.442	1.686	1.220	1.164
20%	1.265	1.265	1.265	1.265
50%	1.686	1.686	1.686	1.686
80%	2.132	2.132	2.132	2.132

ALT, alanine animotransferase; RR, relative risk.

The survival sub-model in Table 5 shows that, the covariates, *age* and *gender,* in the Joint Model indicate similar positive effects on MetS in relation to the covariates in the Cox model (Table 4). Most importantly, the results also reflect a strong positive association between longitudinal augments in *LNALT* and the risk of developing MetS. The results imply a 1 unit longitudinal augment in *LNALT* represents a 3.626-fold (95% CI = 2.721–4.831) increase in the RR of MetS. However, the baseline ALT (*LNALT-0*) is not significant and not retained in the final Joint Mdel.

We estimated the impact of different increments of ALT at specific ALT baseline values on MetS.

Table 6 shows the RRs of MetS that correspond to different longitudinal increments in ALT for different ALT baseline values. For instance, considering the estimated parameter $\hat{\alpha} = 1.288$ in the survival sub-model, a 3 unit enhance in ALT represents a 1.832, 1.402, 1.131 or 1.098-fold increase in the RR of MetS for reference baseline 'true ALT' values 5, 10, 30 or 40, respectively. Therefore, for a particular unit enhancement of ALT, there is a larger impact on MetS risk at a lower baseline ALT activitie. Augments of 20%, 50% or 80% in 5 U/l baseline ALT activities result in RRs around 1.265, 1.686 or 2.132, respectively. However, for the same percent increments in ALT, the RRs are the same across different baseline ALT values. In conclusion, the greater the proportion of dynamic increments of ALT results in the greater incidence risk of developing MetS, regardless of the baseline ALT activities.

4. Discussion

In this large-scale cohort study, the longitudinal associations between ALT and MetS was assessed by the association parameter (α) of the Joint Model. It was suggested that the longitudinal increment of ALT activities over time was associated with an increased risk of developing MetS.

In the present study, the result of the Cox Model showed that the increased risk of MetS was 1.751, which corresponded to a 1 unit augment in baseline *LNALT* (*LNALT-0*). This result is in line with other previous studies. A study with a meta-analysis exhibited that the incidence risk of MetS increased 1.13-fold (95%CI = 1.11–1.16) with 5 U/l increment of ALT [26]. Kunutsor et al. [17] found that the incidence risk of MetS increased by 14% (95% CI = 12%–17%) for every 5 U/l increment in serum ALT activity. A study on ALT and MetS was conducted in a general Japanese population and showed that the odds ratio for the higher quartile of ALT was 4.0 (95% CI = 2.7–6.1), compared with the lower quartile 2.1 (95% CI = 1.4–3.3) in males, and the odds ratio for the higher quartile of ALT was 13.0 (95% CI = 7.3–27), compared with the lower quartile 4.0 (95% CI = 2.1–8.3) in females [27].

However, most of these previous studies only focused on the effects of baseline ALT activities on MetS, which is not consistent with the actual changes of the liver marker. The ALT activity for an individual is not constant. It changes over time with the changing habits of an individual's lifestyle. The liver is the organ central to the functioning of an individual's metabolism. The liver's aging and abnormal accumulation of fat can rupture the hepatocyte, which will cause ALT to be releases into the blood stream. Additionally, unhealthy diets such as those high in fat greatly contribute to liver fat accumulation. A study indicated that serum ALT activity increased with the increment of BMI value after age and sex were accounted for [28]. Iwamoto M et al. conducted a study of dietary intervention and found that serum ALT activity significantly decreased by 20.3% after the subjects consumed low-calorie, well-balanced healthy lunches for a 1-month test period [29]. As present study showed that the average activity of ALT exhibited greater change with follow-up time in MetS group, which is consistent with other study [30]. We suspect that the continuous increment of ALT activities indicates metabolic disorders or disease, including MetS. Therefore, it is necessary to study the relationship between longitudinal increments of ALT and MetS from the perspective of prevention.

The Joint Model result showed that the baseline ALT was not significant when considering the effects of longitudinal increments in ALT activity on MetS and thus was excluded from the final model. This may explain why the effect generated by longitudinal increments of ALT on MetS was greater than that generated by baseline ALT. As we can see form Table 6, a 50% increase in ALT over time for an individual as compared to its baseline level represents a 1.686-fold increased risk of MetS. This result means that the greater the increase in the ALT activity, the greater the occurrence risk of MetS is for an individual, regardless of what their ALT baseline activities are [even the activity within normal reference range ($\leq$40 U/l)]. This result is the most remarkable point of our present study and corresponds with our study aim. However, in most circumstances, a slight increment of serum ALT was hardly to attach awareness and attention for individuals and physicians. This result indicated that over years of continuous health monitoring, we should not only focus on whether the indicators of MetS exceed the normal range, but also continue to stay aware of increasing trends, even trends within the normal reference range.

In this study, we have performed a Joint Model for longitudinal measurements of serum ALT and time to diagnose MetS. Unlike the general Cox Model which only analyzes the baseline data, the Joint Model not only analyzes the baseline, but also the longitudinal increases. Our results are consistent with the literature and clinical knowledge. Our findings indicated that longitudinally elevated ALT for an individual was highly positively associated with the incidence risk of MetS. We also found the risk of MetS in males was higher than that in females and it was became even higher with the increase of age [31,32]. The innovation of our study is that we utilized the longitudinal increments of ALT activities over the follow-up time rather than the baseline. Clinically, it is beneficial to discover the signs of metabolic abnormalities as soon as is possible. Furthermore, the Joint Model makes full use of continuous health check-up data, rather than doing as other studies have done and only focusing health checkups at one specific point in time. The robust association between longitudinal increments in ALT and the incidence of MetS implies that longitudinal increments of ALT play a greater role in the development of MetS than the baseline ALT activity.

The practical significance of our research is that our findings can alert individuals and their primary care physicians to pay more attention to the longitudinal increments of serum ALT during continuous years of physical examinations. A study indicated that about half of the subjects with ALT activities in the upper fourth quartile of the conventional normal range had fatty liver disease that appeared on ultrasonography [33]. That is, when ALT activities for an individual present a certain longitudinal increasing trend, especially beyond the upper quartile of the reference range, it may means an early symptom of metabolism disturbance, such as fatty liver, even MetS. In such a situation the primary care physician should give some advice to the individual regarding his lifestyle habits, such as avoiding the intake of excessive fat, how many hours of sleep the body needs each night, the benefit of physical activities, and so on, so as to protect the individual's liver from damage and ensure serum ALT activities are stable.

There were several limitations in our study. First, most subjects in the present study were young females, which may generate a little bit of bias. Second, the follow-up period was relatively short and only 30.08% subjects were followed-up on for at least 3 or more times, which maybe the reason that the variable of *time* was not statistically significant. Third, there was the lack of WC, as an indicator for central obesity, however, BMI was used as a substitute. Finally, we did not discuss the impacts of other factors on the activities of ALT except for age and gender for the reason that the key point of this study was to reveal the effect of the longitudinal increments in ALT on the incidence risk of MetS. This may cause a little bias. What other factors lead to the increments of ALT activities and how to prevent and control these

increments could be the primary point of research in further studies.

In conclusion, our study demonstrates that a longitudinal increment of serum ALT for healthy Chinese adults, even when ALT remains within the normal reference limits, is an independent predictor of metabolic syndrome. Therefore, carefully monitoring the individual temporal trends in ALT and trying to keep its level stable is a new idea and possible method that could be used to reduce the risk of metabolic syndrome.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.10.033.

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