



## Changes in non-fasting concentrations of blood lipids after a daily Chinese breakfast in overweight subjects without fasting hypertriglyceridemia

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### ABSTRACT

**Background:** Overweight is always accompanied by hypertriglyceridemia (HTG), but the change in non-fasting triglyceride (TG) concentration in overweight subjects without postprandial hypertriglyceridemia was unknown. **Methods:** Concentrations of serum lipids were measured at 2 and 4 h in matched overweight (OW group, n = 54) and control subjects (CON group, n = 55) after a daily meal. Concentrations of remnant cholesterol and non-HDL cholesterol were calculated according to the formulas. The diagnostic criteria for non-fasting HTG were based on 2 different consensus statement. ROC curve was used to determine the pointcut of postprandial HTG. **Results:** OW group had higher fasting concentrations of RC and non-HDL-C than CON group. Non-fasting concentrations of triglyceride and RC significantly increased in 2 groups while were higher in OW group (p < .05). The proportion of non-fasting HTG increased after a daily meal in OW group was significantly higher than the percentage of fasting HTG (p < .05). There was a significant correlation between the postprandial concentrations of TG and RC. **Conclusions:** Overweight subjects were more likely to develop non-fasting hypertriglyceridemia and higher concentrations of RC and non-HDL-C. Additionally, 2.0 mmol/l at 4 h after breakfast could be a pointcut value to detect changes in lipid profile of Chinese overweight people.

### 1. Introduction

The increase in obesity worldwide has a serious influence on public health system and reduces quality of life in individuals [1,2]. Obesity is commonly accompanying with hypertriglyceridemia, both of which are important risk factors to atherosclerotic cardiovascular disease (ASCVD) [3–5]. According to the criterion recommended by Working Group on Obesity in China (WGOC), overweight is defined as body mass index (BMI) between 24 and 27.99 kg/m<sup>2</sup>, general obesity is defined as BMI ≥ 28 kg/m<sup>2</sup> [6]. Though the population of obesity has received much attention, that of overweight is less concerned. Bo et al. [7] showed that from 1993 to 2009 the prevalence of overweight increased from 8.0% to 17.1% among men and from 10.7% to 14.4% among women, respectively, which became an explosive increased type of abnormal BMI. Considering overweight people are also at potentially high risk for ASCVD [8], it should be taken seriously to evaluate

whether serum triglyceride (TG) concentration is normal or not in the overweight population.

According to 2016 Chinese guideline for the management of dyslipidemia in adults [9], the cut-off points of serum TG concentration are as follows: fasting TG concentration ≥ 2.3 mmol/l is defined as increased, i.e., hypertriglyceridemia, < 1.7 mmol/l is defined as desirable, 1.7 mmol/l ≤ fasting TG concentration < 2.3 mmol/l is defined as borderline increased. Among blood lipid parameters, serum TG concentration is greatly influenced by daily diets [10,11]. For human being most of the day is in the postprandial or non-fasting state, several expert panel statements have been proposed to define TG increased or not at randomly time point during the postprandial period [12,13]. Non-fasting TG concentration should not be > 2.0 mmol/l (175 mg/dl) according to a joint consensus statement from the European Atherosclerosis Society (EAS), however, it should not exceed 2.26 mmol/l (200 mg/dl) at any time after a meal according to a scientific statement

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from the American Heart Association (AHA), otherwise, further testing by repeat measurements of fasting TG or a fat tolerance test will be recommended [13,14]. Additionally, hypertriglyceridemia represents increased particles of triglyceride-rich lipoproteins (TRL) and their hydrolyzed products, remnant lipoproteins (RLP) in circulation, especially in the postprandial state [15]. RLP is regarded as atherogenic as low-density lipoprotein (LDL) [16,17], thus the detecting concentration of RLP-cholesterol (RC) is reasonable and necessary. A new method is set up to estimate RC concentration in the non-fasting state after a daily diet, which is signified by total cholesterol (TC) minus LDL-cholesterol (LDL-C) minus high-density lipoprotein-cholesterol (HDL-C) [18], instead of the previously expensive and complicated measurements [19]. Desirable RC concentration is defined as  $< 0.8$  mmol/l in the fasting state and  $< 0.9$  mmol/l in the non-fasting state [12]. Considering the differences in dietary habits, racial and ethnic between the eastern and western populations, it is still uncertain whether the European and American recommendations apply to Chinese subjects, for example, those with overweight.

Currently, research about non-fasting concentrations of blood lipids in Chinese subjects after a daily diet is extremely rare [20], although those after a high-fat diet had been widely reported [21–24]. It was observed that postprandial serum TG concentration reached the peak value at 4 h after a high-fat meal not only in patients with hypertension and coronary heart disease but also in control subjects, although the increment in postprandial TG concentration was obviously different between those patients and controls [23,24], indicating that postprandial serum TG concentration at 4 h could be considered as a surrogate of postprandial hypertriglyceridemia after a high-fat meal [25,26]. However, it is unclear if non-fasting serum TG concentration will reach its peak value at 4 h after a daily breakfast in overweight subjects. According to 2016 Chinese guideline for the management of dyslipidemia in adults [9], the patients with hypertriglyceridemia (fasting TG  $\geq 2.3$  mmol/l) are usually paid more attention than those with borderline increased or desirable TG concentrations unless the latter are found to have hypercholesterolemia. For Chinese subjects with overweight but not hypertriglyceridemia in the fasting state, will their non-fasting TG concentration exceed 2.0 or 2.26 mmol/l after a daily diet? There is no research in this area yet. In this study, we planned to explore the changes in non-fasting concentrations of blood lipids, including TG and RC, after a daily breakfast in overweight subjects and controls without fasting hypertriglyceridemia, and to compare the diagnostic accuracy of hypertriglyceridemia in fasting and non-fasting conditions according to different standards [9,12,13].

## 2. Methods

### 2.1. Subjects

From June 2015 through March 2017, 109 subjects without hypertriglyceridemia in the fasting state were enrolled in the study at the Second Xiangya Hospital of Central South University. All subjects were hospitalized due to supraventricular tachycardia without acute attacks waiting for radiofrequency ablation, or unknown origin of chest pain. They were divided into 2 groups according to BMI: overweight (OW) group with  $24 \leq \text{BMI} < 28$  kg/m<sup>2</sup> and control (CON) group with  $\text{BMI} \leq 18.5$  or  $18.5 < \text{BMI} < 24$  kg/m<sup>2</sup>, according to an expert consensus on the prevention and control of adult obesity in China [27].

Inclusion criteria: (1) fasting serum TG concentration  $< 2.3$  mmol/l, (2) NYHA heart function stage I ~ II class. Excluding criteria: (1) endocrine disease and digestive disease, (2) chronic wasting disease and malignant tumor, (3) TC  $\geq 6.2$  mmol/l, or LDL-C  $\geq 4.1$  mmol/l, (4) taking oral agents for hyperglycemia or hyperlipidemia, (5) Taking  $\beta$ -blockers and diuretics. The research protocol was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. A written informed consent was given by all

participants before the collection of data and conduction of the research.

### 2.2. Specimen collection

All enrolled subjects took the breakfast according to their daily diet habits at 7 am to 8 am after a 12-h overnight fast, and their breakfasts were purchased from the hospital cafeterias or brought home. Venous blood samples were collected before and at 2, 4 h after the breakfast. During the 4-h period, the subjects could drink only water and slow walking until the last blood sample was collected.

### 2.3. Determination of blood lipids

Serum was separated at 4 °C. Serum TC and TG concentrations were measured by enzyme method, HDL-C and LDL-C were measured by chemical masking method [28] on a Hitachi 7170A fully automatic biochemical analyzer by a specialist who was unaware of the study [29]. The reagents were supplied by Japan first joint-stock company. RC and non-HDL-C was calculated by 2 formulas, respectively, i.e.,  $\text{RC} = \text{TC} - (\text{HDL-C}) - (\text{LDL-C})$ , and  $\text{non-HDL-C} = \text{TC} - (\text{HDL-C})$ .

### 2.4. Statistical analyses

Data were analyzed with SPSS (ver. 19.0) and the Graph Pad prism 6.0 software. Quantitative variables were expressed as mean  $\pm$  SE, and qualitative variables were expressed as numbers and percentages. Differences between the intra- and intergroup means were analyzed by unpaired *t*-test or 1-way ANOVA. Categorical variables were compared using chi-squared statistic tests. Coefficients of correlation (*r*) were calculated by Pearson correlation analysis. The optimal cut-off value for non-fasting TG concentration, 2.0 or 2.26 mmol/l, at 4 h was determined using receiver operating characteristic (ROC) curve. Based on the ROC curves, the values determined by the Youden analysis were used as cutoff point. The area under the TG curve (TGAUC) was calculated by the trapezoidal method. TG peak reaction (TGPR) =  $(\text{TG}_2 \text{ h} + \text{TG}_4 \text{ h})/2 - \text{TG}_0 \text{ h}$  (each parameter in equation is the TG concentration of the corresponding time). All *P* values were 2-tailed, and  $p < .05$  was considered statistically significant.

## 3. Results

### 3.1. Baseline characteristics of the study population

The cohort consisted of 70 men (64.2%) and 39 (35.8%) women aged 18–75 y (mean 56 y). Except for BMI, the remained baseline characteristics between CON group and OW group were roughly matched (Table 1).  $< 8\%$  of the subjects had diabetes, and diabetes in the 2 groups were perfectly matched.

**Table 1**

Clinical features of 2 groups of subjects: Numbers are indicated as absolute numbers, percentage and as mean with SD.

	CON (n = 55)	OW (n = 54)
Age [y, SD]	56.9 (13.2)	55.1 (10.5)
M/F, n	35/20	35/19
Current smoking, n (%)	22 (40)	21 (38.9)
Alcohol consumption, n (%)	8 (14.5)	6 (11.1)
Hypertension, n (%)	26 (47.3)	26 (48.1)
Diabetes history, n (%)	7 (12.7)	8 (14.8)
BMI [kg/m <sup>2</sup> , SD]	22.0 (1.3)	26.4 (1.3) <sup>a</sup>

<sup>a</sup>  $p < .01$  for controls vs OW cases.

3.2. The changes in serum concentrations of blood lipids after a daily breakfast

The fasting serum concentrations of non-HDL-C and RC in OW group were significantly higher than those in CON group ( $p < .05$ ). There was nonsignificant difference in the fasting serum concentrations of TC, TG, LDL-C and HDL-C between 2 groups.

After a daily breakfast, there was no significant change in HDL-C concentration when compared with the fasting value. The serum concentrations of TC and non-HDL-C showed a mild tendency of decline, however, that of LDL-C significantly decreased while those of TG and RC significantly increased in the non-fasting state ( $p < .05$ ). Moreover, serum concentrations of TG and RC reached their peak values while serum concentration of LDL-C reached the lowest value at 4 h after a daily breakfast. In this study, the concentrations of LDL-C decreased from 2.26 mmol/l at baseline to 1.84 mmol/l at 4 h in CON group and from 2.45 mmol/l at baseline to 2.01 mmol/l at 4 h in OW group, respectively. The concentration of TG in OW group varied from 1.41 mmol/l at baseline to 2.46 mmol/l at 4 h.

In the non-fasting state, OW group had significantly higher TC concentration only at 4 h, lower HDL-C concentration but higher concentrations of TG, non-HDL-C and RC at both 2 and 4 h than CON group after a daily breakfast ( $p < .05$ ) (Fig. 1). Additionally, both TGPR and TG-AUC were significantly higher in OW group than those in CON group, respectively ( $p < .01$ ) (Supplemental Fig. 1).

3.3. Comparison of the percentages of TG increased according to fasting and non-fasting criteria

The percentage of subjects with fasting TG borderline increased ( $\geq 1.7$  mmol/l) in OW group was higher than that in CON group, but the difference did not reach statistical significance. According to the joint

consensus statement from the EAS [12], the percentage of overweight subjects with non-fasting TG increased ( $\geq 2.0$  mmol/l) at 4 h was significantly higher than that with fasting TG borderline increased ( $p < .05$ ). Moreover, the percentage of subjects with non-fasting TG increased at 2 h or 4 h in OW group was markedly more than that in CON group ( $p < .05$ ) (Fig. 2A).

Although non-fasting TG increased was defined more strictly ( $\geq 2.26$  mmol/l) by the scientific statement of AHA [13], the percentage of subjects with non-fasting TG increased at 4 h in OW group was still significantly higher than that with fasting TG borderline increased in the same group as well as that with non-fasting TG increased at 4 h in CON group ( $p < .01$ ). Within CON group, the percentage of subjects with non-fasting TG increased was less than (at 2 h) or equal to (at 4 h) that with fasting TG borderline increased (Fig. 2B). In order to determine a pointcut of non-fasting TG concentrations at 4 h in relation to fasting TG  $\geq 1.7$  mmol/l, ROC analysis was performed. Youden index was calculated according to the sensitivity and specificity of each possible pointcut in the statistical results, and its maximum pointcut  $pTG = 2.02 \approx 2.0$  mmol/l was selected as the optimal cutoff value (Fig. 2C).

According to the scientific statement from the EAS [12], the abnormal RC concentration is defined as  $\geq 0.8$  mmol/l in the fasting state and  $\geq 0.9$  mmol/l in the non-fasting state. The percentages of subjects with fasting and non-fasting RC increased in OW group were significantly higher than those in CON group ( $p < .05$ ). The percentage of subjects with non-fasting RC increased at 4 h after a daily breakfast was significantly higher than those with fasting RC increased in both CON and OW group ( $p < .05$ ) (Fig. 2D).

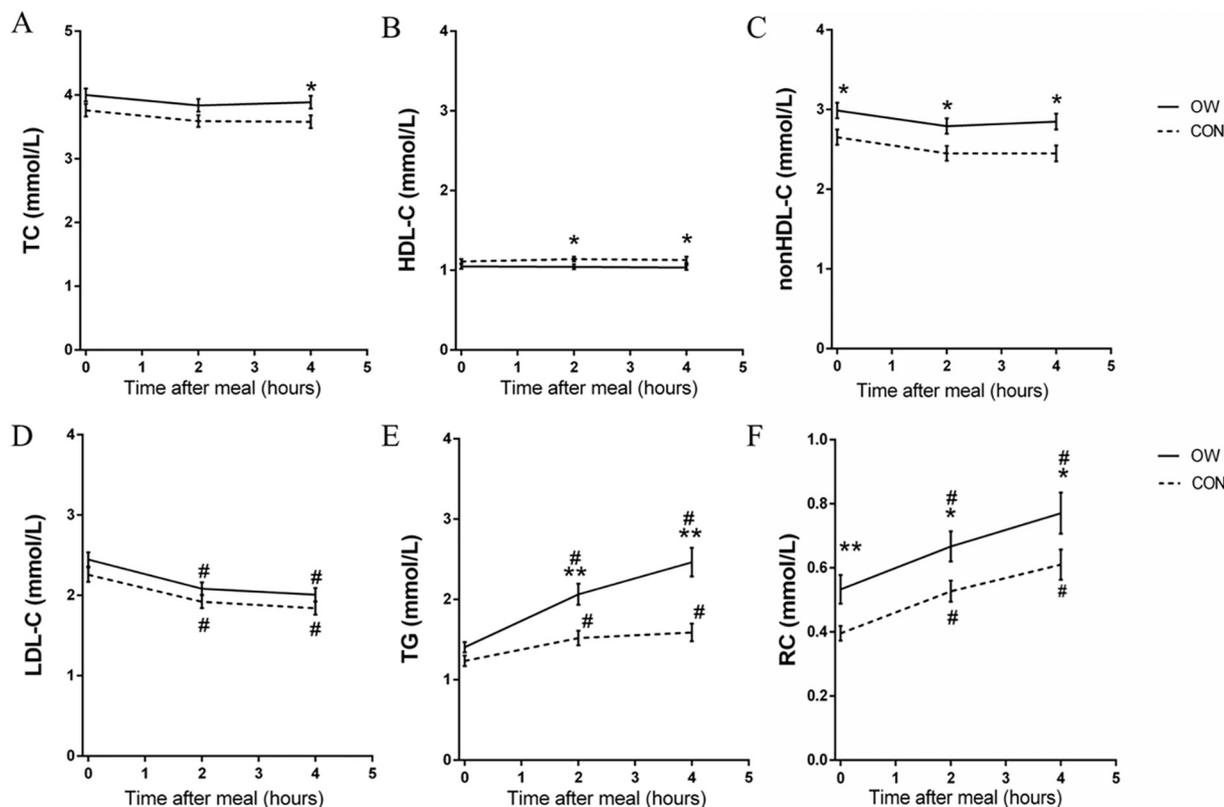
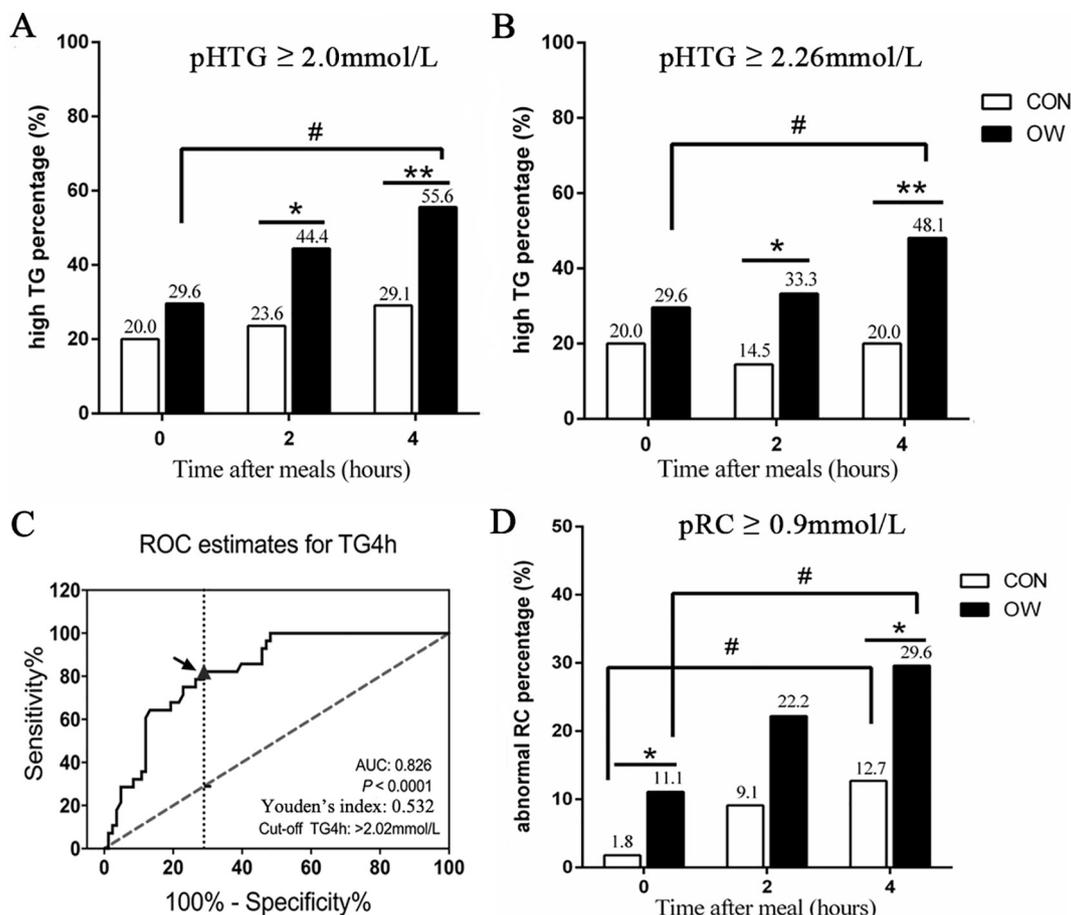


Fig. 1. The changes in serum concentrations of blood lipids after a daily breakfast in 2 groups. (A, B, D, E) The changes in serum concentrations of TC, LDL-C, HDL-C and TG. (C, F) The changes in serum concentrations of non-HDL-C and RC determined by calculated methods. \* $p < .05$  and \*\* $p < .01$  when compared with CON group at the same time point, # $p < .05$  when compared with the fasting concentration in the same group.



**Fig. 2.** The percentages of TG increased and abnormal RC in 2 groups. (A, B) The percentages of fasting vs. non-fasting TG increased in 2 groups by different diagnostic criteria. (C) ROC analysis and Youden's index determines a cutoff point for non-fasting TG concentration, 2.0 vs 2.26 mmol/l, at 4 h in relation to fasting TG  $\geq 1.7$  mmol/l, the cutoff point is indicated by the solid arrow. (D) The percentages of fasting vs. non-fasting abnormal RC in 2 groups. \* $p < .05$  and \*\* $p < .01$  when compared with CON group at the same time point, # $p < .05$  when compared with the fasting concentration in the same group.

### 3.4. Comparison of non-fasting TG concentrations between subjects with different fasting TG concentrations

In the study, it was found that serum TG concentration reached peak value at 4 h after a daily breakfast (Fig. 1E), and the percentage of subjects with non-fasting TG concentration  $\geq 2.0$  or 2.26 mmol/l at 4 h was almost equal to that with fasting TG borderline increased in CON group (Figs. 2A and B). Considering the sensitivity and specificity of the diagnosis, the non-fasting TG concentration  $\geq 2.0$  mmol/l was taken as a cut-point for non-fasting TG increased in the following analysis.

The vast majority of control subjects had not only desirable fasting TG concentrations but also non-fasting TG concentrations  $< 2.0$  mmol/l, while very few control subjects had fasting TG concentrations  $\geq 1.7$  mmol/l and nearly half of them had non-fasting TG increased at 2 h or 4 h ( $\geq 2.0$  mmol/l) (Fig. 3A and C). The number of subjects with desirable fasting TG concentrations and non-fasting TG increased in OW group at 4 h was more than that in CON group at 4 h and that in OW group at 2 h after a daily breakfast. About 30% overweight subjects had fasting TG concentrations  $\geq 1.7$  mmol/l and very few numbers of them had non-fasting TG  $< 2.0$  mmol/l (Fig. 3B and D). It suggested that it is more likely to develop hypertriglyceridemia after daily meals in part of overweight subjects no matter their fasting TG concentrations are desirable or not, especially at 4 h in the non-fasting state.

### 3.5. The correlation between serum concentrations of TG and RC

When taking all subjects as a total whole ( $n = 109$ ), correlation analysis showed that serum TG concentration was positively correlated

with serum RC concentration not only in the fasting state but also in the non-fasting state ( $p < .05$ ). The correlation coefficient at 4 h was higher than that in the fasting state or at 2 h after a daily breakfast (Fig. 4).

## 4. Discussion

This study firstly demonstrated that Chinese overweight subjects had prominently postprandial hypertriglyceridemia after a daily breakfast even their fasting serum TG concentrations were not high, i.e.,  $< 2.3$  mmol/l, suggesting that overweight subjects already have abnormal TRL metabolisms in the non-fasting state before they have fasting hypertriglyceridemia. More importantly, non-fasting serum concentration of RC was higher in OW group than that in CON group, indicating increased risk of ASCVD from RLP particles for overweight in the non-fasting state. It was noticed that there was no significant difference in the fasting serum concentrations of 4 lipids (TC, TG, LDL-C, HDL-C) between 2 groups, and only those of 2 calculated indexes of blood lipids, i.e. non-HDL-C and RC, increased significantly in subjects with overweight. Thus, for overweight subjects with borderline increased or even desirable TG, it is not enough to merely analyze routine four lipids in the fasting state.

It has been known that estimated RC in the fasting state represents cholesterol within very low-density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) [30]. As the main components of postprandial RC, the latter 2 together with cholesterol within chylomicrons constitute RC in the non-fasting state [31]. After nascent chylomicrons and VLDL are successively secreted into the circulation, TG

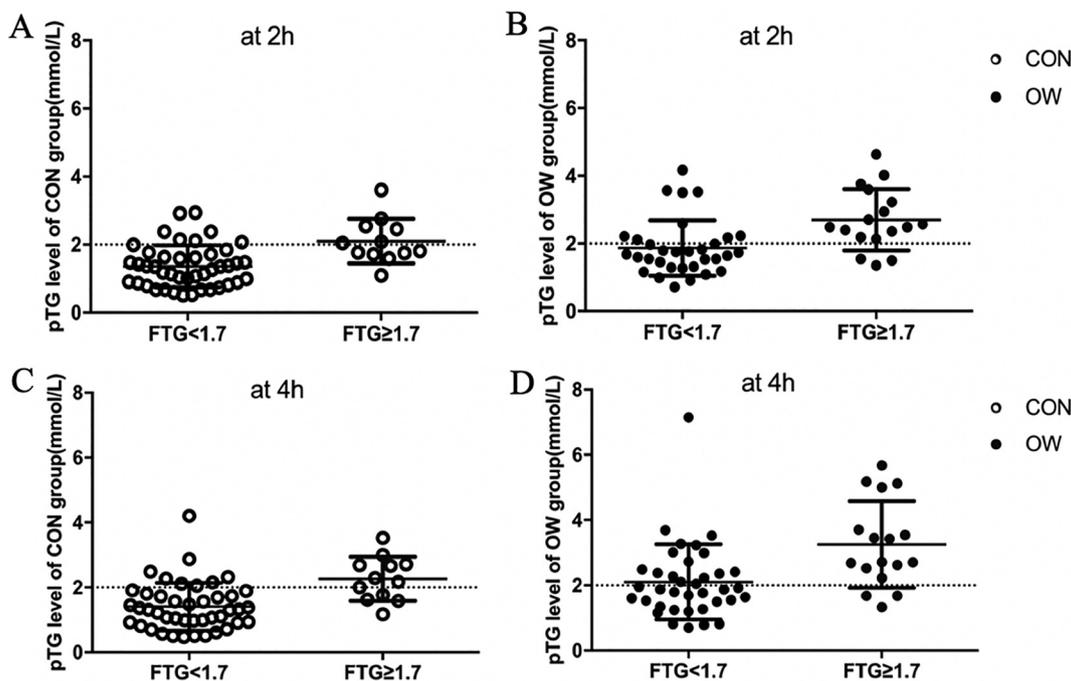


Fig. 3. The comparison of non-fasting TG concentrations between 2 groups according to different fasting TG concentrations. Postprandial TG (pTG) concentrations at 2 h (A and B) and 4 h (C and D) after a daily breakfast in CON group and OW group, the subjects in the 2 groups divided by fasting TG concentration <math>< 1.7</math> or not. Solid circles represent OW group, and open circles represent CON group.

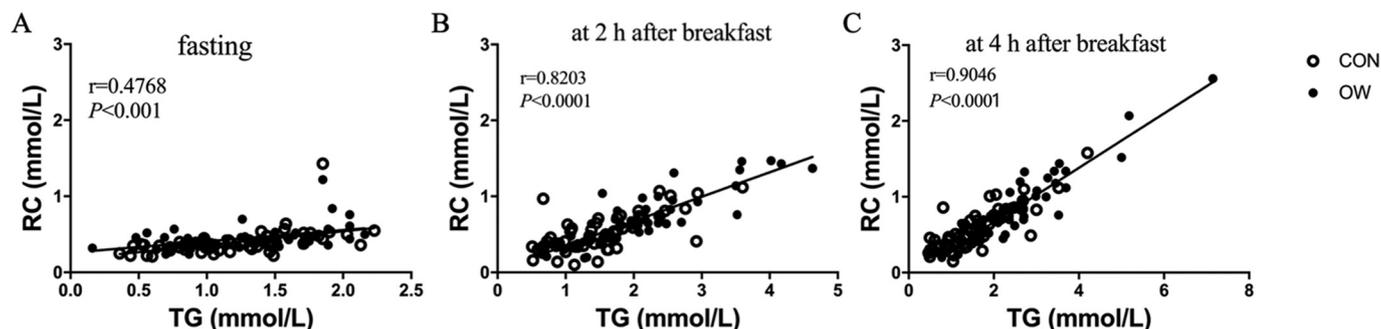


Fig. 4. The correlation between concentrations of RC and TG in the fasting and non-fasting state. (A) The correlation between serum concentrations of RC and TG in the fasting state. (B–C) The correlation between serum concentrations of RC and TG at 2 h (B) and 4 h (C) after a daily breakfast. Solid circles represent OW group, and open circles represent CON group.

within TRL would be rapidly hydrolyzed by lipoprotein lipase. Therefore, TRL can be basically equivalent to RLP, except for those subjects with complete deficiency in lipoprotein lipase [32]. That could be the reason for high correlation coefficient between TG and RC in the non-fasting state.

In this study, we focused on the subjects with OW, a group of apparently “healthy” people, but not obese individuals, although they may share the same pathogenic mechanism. Remnant lipoproteins (RLP) in plasma are significantly increased as the result of disturbed lipoprotein metabolism followed by obesity, abnormal adipocytokines secretion and insulin resistance [33–35]. Firstly, scavenging rates of TRL and RLP could slow down in subjects with overweight/obesity. Yuka et al. [36] found that the obese men showed significantly delayed TRL metabolism compared to the young men after fat loading, because of the decrease of LPL activity caused by insulin resistance. Secondly, assembly and secretion of TRL, including VLDL and chylomicrons, increase in the liver and intestine [37]. It was partly supported by higher TGPR and TG-AUC in OW group (See Supplemental Fig. 1). Thirdly, uptake and utilization of TRL and RLP by peripheral tissues are impaired for the reduced concentration of adiponectin and the increased concentration of glucagon-like peptide-1 (GLP-1) in overweight and

obese subjects [35,38,39]. Therefore, lifestyle changes especially body fat reduction through exercise and diet control should be effective measures to treat postprandial hypertriglyceridemia in overweight individuals.

Comparing with obvious changes in TG and RC, serum concentration of HDL-C was stable in the non-fasting stated and that of TC or non-HDL-C showed a tendency of mild reduction that did not reach statistics significance, which is in line with the results of a previous study about the general population of Denmark [40]. It is surprising that reduction in non-fasting LDL-C concentration in 2 groups was > 0.2 mmol/l while increment in non-fasting TG concentration of OW group was > 0.3 mmol/l reported by Danish researchers [40], although the causes for the non-fasting reduction in LDL-C were complicated and controversial. Compared with the westerners, the traditional Chinese diet has a high carbohydrate content, such as steamed bread, noodles, vermicelli, rice porridge, steamed stuffed bun and so on, but not breakfast rich in protein and/or fat, such as cheese, sausage, ham and bacon [41,42]. No investigation was found on comparing Chinese with European after a high-carbohydrate meal, although the definition of high-carbohydrate meal between studies in Chinese and European varies slightly [43–46]. The study about changes in blood lipids after a high-carbohydrate meal

in Chinese was rare. It is worth noting that there was no significant increase in postprandial triglyceride concentrations in healthy Europeans as well as Asians, such as Koreans, after a high-carbohydrate meal without fat [47,48]. The differences in dietary habits, sample number, disease status and race among different studies could be associated with more obvious reduction in LDL-C and increment in TG. In addition, as the sum of LDL-C and RC, non-HDL-C represents cholesterol content within nearly all atherogenic lipoproteins in circulation [49].

In the present study, we tried to diagnose non-fasting TG increased (i.e., postprandial hypertriglyceridemia) according to 2 different cut-points, i.e., 2.0 and 2.26 mmol/l, recommended by the EAS and AHA, respectively. No matter which cut-off point was taken, the percentage of overweight subjects with postprandial hypertriglyceridemia was consistently higher than that with fasting TG borderline. More than half subjects with overweight had postprandial hypertriglyceridemia at 4 h after a daily breakfast. More importantly, even with desirable fasting TG concentration, overwhelming overweight subjects had non-fasting TG concentration  $\geq 2.0$  mmol/l at 4 h after breakfast. These findings indicated that the diagnosis of hypertriglyceridemia could be more dependent on non-fasting TG concentration but not fasting TG concentration for overweight subjects.

In the Copenhagen city heart study, the non-fasting blood lipids were randomly detected in the large population of Denmark [50]. In that situation, the detection of non-fasting blood lipids is equivalent to measure random blood glucose. Unlike blood glucose testing, the peak-value time point of TG after a high-fat diet or daily diet is at 4 h but not at 2 h in the Chinese [24]. Therefore, for the individual with a certain disease, such as overweight, obesity, diabetes, hypertension or coronary heart disease, 4 h after a high-fat or daily meal can be selected as an appropriate time point to observe the changes in non-fasting concentrations of blood lipids, especially TG and RC in clinical practice, and a comprehensive judgment of hypertriglyceridemia should be made in combination with the concentrations of blood lipids in the fasting and non-fasting states [14].

There were some limitations in this study. Firstly, some subjects bought breakfasts in the hospital canteen, which could be different from their usual diets at home. Secondly, the number of samples was relatively small. Thirdly, the detection of hypertriglyceridemia in the non-fasting state was only evaluated in the subjects with overweight or not. For patients with diagnosed ASCVD and other diseases, it is also necessary to observe the concentrations of blood lipids in the non-fasting state in the future.

In conclusion, our study highlighted that overweight people without fasting hypertriglyceridemia were more likely to develop postprandial HTG. Furthermore, we thought that the concentration of TG at 4 h after a meal, 2.0 mmol/l, could be an appropriate pointcut value to detect changes in lipid profile of overweight people in China. However, further studies with larger sample assessing the predictive usefulness of postprandial TG and remnant cholesterol for Chinese overweight/obese people need to be considered.

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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.2019.01.004>.

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