

Generating a reference interval for fasting serum insulin in healthy nondiabetic adult Chinese men

Shan Li¹, MD, Shan Huang¹, MD, Zeng-Nan Mo^{2,3}, MD, PhD, Yong Gao³, MD, Xiao-Bo Yang⁴, PhD, Xue-Jie Chen¹, MD, Jin-Min Zhao⁵, MD, Xue Qin¹, MD

INTRODUCTION Circulating insulin concentrations provide important information for the evaluation of insulin secretion and insulin resistance. Reference intervals are the most widely applied tool for the interpretation of clinical laboratory results. We carried out an analysis of the data available from the Fangchenggang Area Male Health and Examination Survey in order to derive a reference interval for fasting insulin specific to the Chinese population.

METHODS A total of 1,434 fasting serum insulin results were obtained from healthy nondiabetic adult men aged 20–69 years, after taking into consideration the inclusion and exclusion criteria. Serum insulin was measured using electrochemiluminescence immunoassays. Nonparametric statistical methods were used to calculate and analyse the data.

RESULTS The reference interval for fasting serum insulin for Chinese adults was in the range 1.57–16.32 $\mu\text{U/mL}$ (median 5.79 $\mu\text{U/mL}$). Significant correlations were found between fasting serum insulin and glucose and diastolic blood pressure ($p < 0.001$). Statistically significant differences were observed in insulin concentration with respect to age and body mass index (BMI; $p < 0.001$). Younger people had a higher fasting serum insulin concentration. Increased fasting serum insulin was also found to be associated with BMI.

CONCLUSION We established a reference interval for fasting serum insulin in healthy nondiabetic adult Chinese men that is lower than what was previously suggested. BMI and age (but not smoking, alcohol consumption or physical activity) were found to be important factors associated with fasting serum insulin. Our results will help improve the diagnostic interpretation of investigations for metabolic and cardiovascular disorders in a Chinese population.

Keywords: *elecsys assay, fasting insulin, male, metabolism, reference interval*
Singapore Med J 2012; 53(12): 821–825

INTRODUCTION

Insulin is well known as a metabolic hormone that mediates glucose uptake. However, insulin also affects vascular tone, which largely depends on its ability to stimulate the synthesis and release of endothelial mediators, and whose balanced activity ensures dynamic control of vascular function.⁽¹⁾ Therefore, circulating insulin concentrations and resistance to insulin action have been proposed as potential common factors linking metabolic and cardiovascular disorders.⁽²⁾

Reference intervals are the most widely applied tool for the interpretation of clinical laboratory results. Various studies have determined the reference interval for fasting serum insulin in adults from different countries using different detection methods and obtained diverse results.^(3–6) However, there is a lack of published data regarding the reference interval for fasting serum insulin in the Chinese population. Additionally, few laboratories have conducted their own reference interval studies.⁽⁷⁾

China, the world's most populous country, has enjoyed impressive economic development over the past two decades.⁽⁸⁾ The Chinese have experienced many remarkable changes in their lifestyles due to both an increase in family income as well as the increased availability of food owing to increased global

trade and advances in agriculture. However, such changes in human behaviour and lifestyle have resulted in an increase in the incidence of metabolic and cardiovascular diseases in China, which are preventable causes of death. As concerns and discussions about the health of men increase around the world, governments are formulating policies aimed at improving their health.⁽⁹⁾

In the light of these realities, we carried out an analysis of the data available from the Fangchenggang Area Male Health and Examination Survey (FAMHES)⁽¹⁰⁾ in order to derive a reference interval for fasting insulin specific to the adult Chinese male population. Our study was carried out according to standard operating procedures advocated in document C28-A3 of the Clinical and Laboratory Standards Institute (CLSI)/National Committee for Clinical Laboratory Standards (NCCLS)⁽¹¹⁾ for a Chinese population.

METHODS

Data on fasting serum insulin was obtained from the FAMHES.⁽¹⁰⁾ Fangchenggang, a city of about 800,000 in southern China, is a port city of the Association of Southeast Asian Nations (ASEAN) free trade zone. The FAMHES was a population-based study of noninstitutionalised Chinese men between 17–88 years of age.

¹Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, ²Institute of Urology and Nephrology, First Affiliated Hospital of Guangxi Medical University, ³Center for Genomic and Personalized Medicine, Guangxi Medical University, ⁴Department of Occupational Health and Environmental Health, School of Public Health of Guangxi Medical University, ⁵Department of Orthopaedic Trauma Surgery, First Affiliated Hospital of Guangxi Medical University, Guangxi, China

Correspondence: Dr Xue Qin, Vice Director, Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China. qinxue919@yahoo.cn

It was designed to investigate the effects of environmental and genetic factors, and their interactions with the development of age-related chronic diseases. A comprehensive demographic and health survey was conducted on 4,303 men who participated in a large-scale physical examination at the Fangchenggang First People's Hospital Medical Centre, Fangchenggang, China, from September 2009 to December 2009.

Participants were physically examined by doctors prior to undergoing laboratory screening. The inclusion criteria were as follows: (a) a stable healthy lifestyle; (b) reasonably healthy men; (c) fasting blood glucose level < 5.6 mmol/L and no previous diagnosis of type 2 diabetes mellitus; and (d) not obese (body mass index [BMI] < 28 kg/m²). Exclusion criteria specified in the CLSI guidelines⁽¹¹⁾ were used to exclude participants who met the following descriptions in our database: (a) chronic disorder requiring regular medication; (b) diabetes mellitus, hypertension, coronary heart disease, stroke, tuberculosis, liver cirrhosis, cancer, hyperthyroidism, rheumatoid arthritis, systemic lupus erythematosus, chronic bronchitis, or liver/kidney failure; (c) recovering from surgery (< 14 days) or acute illness; (d) intake of prescribed drugs during the preceding two weeks; (e) donated blood in the preceding five months; (f) consumed more than two measures of alcohol (a measure is equal to 12 g of alcohol) in the preceding 24 hours; or (g) heavy smoker (> 20 cigarettes/day). From 4,303 participants, 1,434 healthy nondiabetic adult men were enrolled in our study (age range 20–69 years). All participants provided written informed consent and the study was approved by the local ethics committee.

Trained physicians conducted face-to-face interviews with the participants. Information was collected using a standardised questionnaire on demographic data (such as age, occupation, etc.), health status, medical history and lifestyle characteristics (such as smoking, alcohol consumption and physical activity). Participants were categorised based on age into three groups: 20–44 years (youth), 45–59 years (middle-aged) and 60–69 years (elderly). Physical activity level was classified as low, moderate or high, according to the questionnaire's scoring protocol.⁽¹²⁾ BMI was calculated as weight/height² (kg/m²) and used as an indicator of obesity. Participants were categorised as normal (< 24.0 kg/m²), overweight (24.0–27.9 kg/m²) or obese (≥ 28.0 kg/m²) based on their BMI.⁽¹³⁾ Obese participants were excluded from the analysis.

Blood samples were collected between 8:00 am and 10:30 am following an overnight fast. 10 mL of blood was drawn from each participant using evacuated serum separator blood collection tubes. Each participant was given a unique sample number that would be used to label the samples and tied to the analytical results. Blood samples were transported at low temperatures to the clinical laboratory at the First Affiliated Hospital of Guangxi Medical University, Nanning, China. The samples were kept at room temperature for 10 minutes before centrifugation for 15–25 minutes at a minimum of 1,500 g. The serum was stored at -80°C until analysis. Parameters such as

fasting serum glucose were obtained in the Fangchenggang area laboratory and measured enzymatically using an automatic analyser (Dade Behring, Newark, NJ, USA). Fasting serum insulin was measured using electrochemiluminescence immunoassays on an immunoassay analyser (Roche Cobas[®] 6000 system E601 Elecsys module; Roche Diagnostics GmbH, Mannheim, Germany) with the same batch of reagents. The reconstituted quality control material, Elecsys PreciControl tumour markers, provided by Roche Diagnostics, was measured in each batch of analysis as control according to the manufacturer's instructions. The interassay variation coefficients ranged between 3.2%–4.8%.

Outliers were detected in the selected data by means of the Dixon outlier range statistic, which was recommended in the document C28-A3 of the CLSI/NCCLS guidelines.⁽¹⁴⁾ Finally, one outlier, which was found to have a fasting serum insulin of 63.90 $\mu\text{U/mL}$, was excluded from further calculations. Data were analysed using the Statistical Package for the Social Sciences for Windows version 17.0 (SPSS Inc, Chicago, IL, USA), unless otherwise stated. The one-sample Kolmogorov-Smirnov (K-S) test was used from among the nonparametric tests to test distribution normality. When no distribution normality was found, nonparametric statistical methods were used to calculate and analyse the data. Insulin level distributions were presented as median, and the reference interval was obtained from the 2.5th–97.5th percentile range. Spearman's partial correlation coefficients of fasting serum insulin and risk factors were calculated. A nonparametric Kruskal-Wallis test was used to compare fasting serum insulin values by age, BMI and physical activity levels. Differences in fasting serum insulin levels across smokers or drinkers were evaluated using the Mann-Whitney U test. Analysis of covariance was done to investigate the effects of age and BMI on other correlative covariates. A p -value < 0.05 on the two-sided test was considered statistically significant.

RESULTS

Table I presents the characteristics of the participants in our study. The fasting serum insulin concentrations ranged from undetectable levels to 41.14 $\mu\text{U/mL}$. The values were not normally distributed (K-S test; $p < 0.05$). The reference interval for healthy, nondiabetic Chinese men in the present study was in the range 1.57–16.32 $\mu\text{U/mL}$ (median 5.79 $\mu\text{U/mL}$).

Partial correlation analysis (adjusted for age and BMI) showed that fasting serum insulin concentrations were significantly associated with glucose and diastolic blood pressure (all $p < 0.001$; Table I). There was a significant difference in fasting serum insulin levels across smoking statuses ($Z = -3.446$; $p < 0.001$), BMI categories ($\chi^2 = 260.588$; $p < 0.001$) and age groups ($\chi^2 = 16.055$; $p < 0.001$). Interestingly, a small but statistically significant difference was found between younger participants and the other age groups after adjusting for BMI ($F = 6.399$; $p < 0.001$), indicating that younger individuals had higher insulin concentrations (Table II). It was also observed that lower BMI tended to lower insulin concentrations (Fig. 1), even when

Table I. Participant characteristics and correlation with fasting serum insulin levels (n = 1,434).

Parameter	Median	Quartile	Range	Spearman r	Partial r (adjusted for BMI)	Partial r (adjusted for BMI and age)
Age (yrs)	35	28–42	20–69	-0.102*	-0.146	-
Weight (kg)	63.5	57.0–69.5	43.0–90.0	0.443*	0.068*	0.048
Height (cm)	168.0	164.4–172.0	152.0–185.7	0.037	0.061*	0.039
BMI (kg/m ²)	22.0	20.0–24.0	15.8–27.9	0.473*	-	-
Fasting glucose (mmol/L)	5.1	4.8–5.3	3.0–5.6	0.104*	0.068*	0.087*
Systolic blood pressure (mmHg)	116	106–123	85–139	0.123*	-0.007	0.033
Diastolic blood pressure (mmHg)	76	70–80	50–102	0.165*	0.057*	0.080*

*p < 0.05

BMI: body mass index

Table II. BMI- and age-dependent reference intervals for fasting serum insulin.

Parameter	Fasting serum insulin reference interval					F	p-value*
	No. of participants	Median	2.5th–97.5th percentile	Quartile interval			
Body mass index (kg/m²)						136.288	< 0.001
< 17.99	55	4.68	1.15–19.59	2.90			
18–23.99	924	5.00	1.33–13.42	3.31			
24–27.99	455	8.18	3.11–19.29	5.01			
Age (yrs)						6.399	< 0.001
20–44	1,142	5.91	1.73–15.96	4.20			
45–59	226	5.08	1.21–19.04	4.44			
60–69	66	5.04	0.90–22.90	5.38			
Total	1,434	5.79	1.57–16.32	4.35			

*Statistically significant differences were found for fasting insulin levels between the body mass index and age groups, respectively, on covariate analysis, once adjusted for age or body mass index.

adjustments were made for age ($F = 136.288$; $p < 0.001$) (Table II). In contrast, there was no difference between smoking and non-smoking participants when adjusted for BMI and age ($F = 2.132$; $p = 0.145$). No significant differences were observed between the participants based on drinking ($p = 0.253$) and physical activity ($p = 0.920$).

DISCUSSION

The present study analysed data collected during the 2009 FAMHES study,⁽¹⁰⁾ with the objective of deducing a reference interval for fasting serum insulin levels using electrochemiluminescence immunoassays in healthy nondiabetic adult Chinese men in accordance with the CLSI/NCCLS C28-A3 guidelines.⁽¹¹⁾ Four inclusion and seven exclusion criteria were used to comprehensively define health status among the participants due to difficulties in validating healthy individuals otherwise. As outliers might have existed in specimens from healthy people, leading to lower accuracy of the endpoint estimators of the reference interval, these were excluded from the database. Data on women were not included in our investigation. However, this limitation was compensated by the fact that no gender-based differences in fasting serum insulin levels have been reported thus far among men and women.^(15–18) All measures mentioned above were intended to minimise errors and render the results more reliable.

The two-sided nonparametric reference interval for fasting serum insulin in men, as calculated from a total of 1,434 healthy nondiabetic adult men in our study, was 1.57–16.32 $\mu\text{U/mL}$

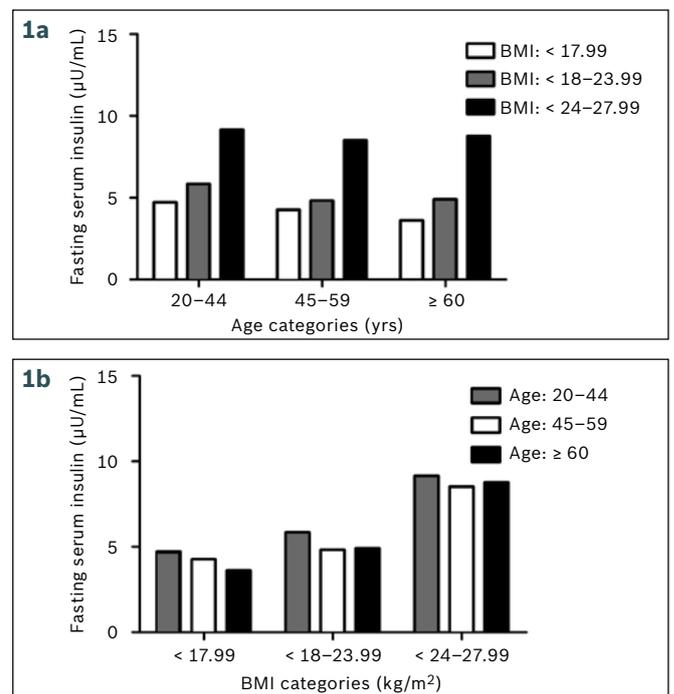


Fig. 1 Graphs show the median values of fasting serum insulin with respect to (a) age and (b) body mass index (BMI). Fasting serum insulin concentrations were positively correlated with BMI ($r = 0.473$; $p < 0.001$) and negatively correlated with age ($r = -0.102$; $p < 0.001$). Young men had higher fasting serum insulin, but a small statistically significant difference was seen for all BMI groups. Insulin tended to increase with rising BMI in every age group.

(median 5.79 $\mu\text{U/mL}$). Interestingly, the reference values obtained for fasting serum insulin in our study were significantly lower than those reported in previous studies and investigations, which

obtained varying results ranging from the detection limit to 26 $\mu\text{U/mL}$.^(3,4) Some studies have investigated fasting serum insulin with a focus on ethnic differences. For instance, a systematic review and meta-analysis by Takeuchi et al that investigated fasting serum insulin levels in East Asian participants reported that these levels were significantly lower in Japanese patients when compared to Korean and Chinese patients.⁽¹⁹⁾ Osei et al indicated that fasting serum insulin in related African-American individuals was significantly higher than in related Nigerian natives.⁽²⁰⁾ Similarly, a comparative study of three populations showed that the mean fasting insulin concentration was significantly higher in native Ghanaians than in African-Americans, Ghanaian immigrants and white Americans.⁽²¹⁾

We found that it was difficult to interpret the reference values supplied by commercial assay kits due to the limited amount of clinical information provided with them, which is usually based on studies conducted with small sample sizes. The reference interval provided by the manufacturer for fasting serum insulin levels (range 2.6–24.9 $\mu\text{U/mL}$; from the 5th–95th percentile range) was derived from studies conducted in a German clinical centre with samples from 57 healthy and fasting individuals.

Based on these results, we inferred that significant differences were likely to exist between the results of earlier studies and our study, which may be due to a number of reasons. Firstly, participants in our study were from a Chinese population living in the coastal area of Guangxi, China, whereas participants of previous studies were from Nordic countries, the USA, Nigeria, Ghana, Japan and Korea, among others. The disparity in the findings of these studies may therefore reflect the differences among diverse ethnic groups, and the lifestyles and dietary habits of different populations. Secondly, it is possible that other studies may have used less reliable assays and/or smaller samples.⁽²²⁾ In our study, the Roche Cobas® 601 detection system was used for electrochemiluminescence immunoassays, which was different from the systems used in other studies.^(23–25) Differences in study populations and measurement tools may thus largely be responsible for the inconsistencies seen in the results of the various studies.

Unlike findings in children,⁽²⁶⁾ the present data indicated an inverse relationship between fasting serum insulin and age in nondiabetic adult men. On analysis of covariance, fasting insulin levels were significantly different in the different age groups after adjustment for BMI. Younger participants had higher fasting insulin concentrations, and insulin secretion decreased with age. It is possible that the degree of insulin secretion or glucose intolerance in the elderly may be related to pancreatic dysfunction that persists even after improvements are made in their lifestyles.⁽²⁷⁾ The small effect of age on insulin action may thus be explained in terms of age-related changes to body composition and substrate competition.⁽²⁸⁾ We inferred that in healthy nondiabetic Chinese men, age would be a significant factor for insulin metabolism.

Physical activity provides several benefits, and the type and intensity of activity are associated with improvements in health

and quality of life.⁽²⁹⁾ Physical activity increases sensitivity to insulin, which is recommended for patients with noninsulin-dependent diabetes mellitus.⁽³⁰⁾ In our study, participants had stable and healthy lifestyles, and most participants were young, with comparable physical activity and diet. Therefore, in the healthy state, there was no statistically significant difference among participants from the various physical activity groups.

Tobacco and alcohol consumptions, which are very common around the world, may increase the risk of obesity by causing insulin resistance.^(31–33) Participants in our study were classified as non-smokers or smokers and non-drinkers or drinkers, based on their smoking or drinking status. No significant difference was found between participants from the drinking and non-drinking groups. Although on univariate analysis, a difference was found between the smoking and non-smoking participants in our study, this difference was not significant after adjustment for BMI. Smoking has been shown to have an effect on glucose tolerance and insulin sensitivity. According to Balkau et al, men who cut smoking over three years were associated with significant increases in fasting glucose and insulin levels.⁽³⁴⁾ Several studies also report that smokers have a lower BMI than non-smokers^(35,36) and that people are likely to gain weight if they quit smoking.⁽³⁷⁾ Perkins suggested that nicotine intake from smoking may increase metabolic rate (as the primary mechanism) rather than decrease energy intake due to appetite suppression.⁽³⁸⁾ It is possible that smokers use energy faster, and thus a lower insulin resistance may result in lower fasting insulin levels in smokers. Due to its effect on BMI, it is unlikely that the effect of smoking on insulin may play a direct role in diabetes mellitus.

In a cross-sectional study, Hojlund et al reported that a higher BMI resulted in higher fasting insulin levels.⁽³⁹⁾ The results of our study agree with this finding, as we found that there was a significant correlation between fasting serum insulin levels and BMI, independent of age, i.e. fasting serum insulin levels gradually increased with increasing BMI. In other words, individuals with high BMI were associated with higher fasting insulin levels. Obesity, which is a known risk factor for metabolic and cardiovascular disorders, is now common in China. Owing to its association with adiposity, low cost and ease of measurement, BMI has been suggested as the preferred clinical and epidemiologic surrogate for predicting coronary heart disease.⁽⁴⁰⁾ In keeping with these findings, we propose that BMI-dependent reference intervals for fasting serum insulin levels be implemented in clinical laboratories.

In the present study, we established the reference interval for fasting serum insulin in healthy nondiabetic adult Chinese men. Our reference interval for fasting serum insulin was lower than those suggested by previous studies and investigations, and correlated with glucose levels and diastolic blood pressure. BMI and age, but not smoking, drinking or physical activity, were important factors for fasting serum insulin. Our findings will help improve the diagnostic interpretation of investigations of metabolic and cardiovascular disorders.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (grant no. 30945204) and the provincial departments of finance and education, Guangxi Zhuang Autonomous Region, China (grant no. 2009GJCJ150). We are grateful to the local research teams from Fangchenggang First People's Hospital, Fangchenggang, China, for their contribution to the survey. No potential conflicts of interest were relevant to this article.

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