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Analysis of influencing factors of serum total protein and serum calcium content in plasma donors

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Background and objectives: The adverse effects of plasma donation on the body lowered odds of donation. The aim of this study was to investigate the prevalence of abnormal serum calcium and total serum protein related to plasma donation, identify the influencing factors, and come up with suggestions which can make plasma donation safer. **Methods:** Donors from 10 plasmapheresis centers in five provinces of China participated in this study. Serum calcium and total serum protein of samples which were collected before donation were measured by automatic biochemical analyzer. **Results:** The mean serum calcium was 2.3±0.15 mmol/L and total serum protein was 67.75±6.02 g/L. The proportions of plasma donors whose serum calcium and total serum protein were lower than normal were 20.55% (815/3966) and 27.99% (1111/3969), respectively. There were significant differences in mean serum calcium and total serum protein of plasma donors with different plasma donation frequencies , gender, age, regions, and body mass index (BMI), (all *p*□0.05). Logistic regression analysis revealed that donation frequencies, age, BMI and regions were significantly associated with a higher risk of low serum calcium level, and donation frequencies, gender, age and regions were significant determinants factors of odds of abnormal total serum protein. **Conclusions:** Donation frequencies, gender, age, regions, and BMI showed different effects on serum calcium and total serum protein. More attention should be paid to the age, donation frequency and region of plasma donors to reduce the probability of low serum calcium and low total serum protein.

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Abstract

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Methods: Donors from 10 plasmapheresis centers in five provinces of China participated in this study. Serum calcium and total serum protein of samples which were collected before donation were measured by automatic biochemical analyzer.

Results: The mean serum calcium was 2.3±0.15 mmol/L and total serum protein was 67.75±6.02 g/L. The proportions of plasma donors whose serum calcium and total serum protein were lower than normal were 20.55% (815/3966) and 27.99% (1111/3969), respectively. There were significant differences in mean serum

calcium and total serum protein of plasma donors with different plasma donation frequencies, gender, age, regions, and body mass index (BMI), (all $p<0.05$). Logistic regression analysis revealed that donation frequencies, age, BMI and regions were significantly associated with a higher risk of low serum calcium level, and donation frequencies, gender, age and regions were significant determinants factors of odds of abnormal total serum protein.

Conclusions: Donation frequencies, gender, age, regions, and BMI showed different effects on serum calcium and total serum protein. More attention should be paid to the age, donation frequency and region of plasma donors to reduce the probability of low serum calcium and low total serum protein.

Keywords: plasma donor, influencing factors, serum calcium, total serum protein.

1 Introduction

As important medicines for clinical use, blood and blood components save millions of lives each year and are included in the Model List of Essential Medicines of the World Health Organization (WHO) [1]. Plasma-derived medicinal products (PDMPs), such as albumin, coagulation factors and immunoglobulins, are prepared from human plasma. They are used to prevent and treat a variety of life-threatening diseases [2]. The statistical results of WHO in 2020 showed, only 56 of 171 reporting countries produced PDMPs through the fractionation of plasma, and 91 countries reported that all PDMPs were imported [3]. The risk for PDMPs shortages as well as increasing demand may result in depriving patients of essential medicinal products [4].

Source plasma (SP) the raw material of PDMPs and the most challenging aspect of manufacturing PDMPs. And it is considered as a strategic resource. Most SP (85%-90%) is collected by apheresis from donors and a small fraction (10-15%) is contributed by recovered plasma [5]. In China, all SP is obtained from apheresis plasma. Donation serves the demand for plasma but there are concerns among potential donors about the impact of blood loss on physical health. Concerns about the health impact of plasma donation limit the number of plasma donations [6].

It is reported that donors perceive both positive and negative effects of blood donation [7].

The most often reported positive symptoms are: alleviated headache, feeling lighter, and less tiredness [7-10]. And the most common negative effects are iron deficiency, vasovagal reactions and citrate-related events [11]. Most researches which studied the effects of blood donation or plasma donation on the health of donors focused on the types of adverse reactions. Little attention is paid on the influencing factors leading to these adverse reactions. In order to further understand the impact of plasma donation on donors and to come up with suggestions which can make voluntary plasma donation safer, we investigated the changes of serum calcium and total serum protein in different types of plasma donors. The factors which affecting serum total protein and serum calcium were also analyzed.

2 Material and methods

2.1 Sample Collection

A total of 4000 subjects (2000 male and 2000 female) were recruited for this study. All participants were healthy plasma donors who donated during Jun. 25, 2021 to Sep.4, 2021, and came from 10 plasmapheresis centers in five provinces (Sichuan, Jiangxi, Hubei, Shandong, and Ningxia) of China. The inclusion criteria were that all participators were 18-60 years, healthy, and unrelated. People who had history of thrombus or hemorrhage, usage of oral anticoagulation therapy, pregnancy, HIV infection, hepatic disease, diabetes, renal insufficiency and etc. were excluded from this research.

Five microliter venous blood of each participant was collected in a sterile tube without anticoagulant before plasmapheresis. After clot formation, the samples were centrifuged at 3,000 g for 10 min, removed serum and then immediately stored at -70 °C in two aliquots until being transported to institute of blood transfusion (IBT) on dry ice. Once arrived IBT laboratory, one aliquot was immediately used for serum calcium detection, and the other aliquot was used for total serum protein detection.

After excluding incomplete information, duplicate and data drift samples, 3966 and 3969 subjects were available in the statistical analysis of serum calcium and total serum protein, respectively. The demographic information of participants was shown in Table 1.

2.2 Laboratory assays

Serum calcium and total serum protein were performed using an automatic biochemical analyzer (Beckman Coulter AU5800; Beckman Coulter Inc., Brea CA, USA) according to the manufacture's protocols. Serum calcium and total serum protein reagents were purchased from Beckman Coulter, Inc. (Brea CA, USA).

2.3 Statistical analysis

Kolmogorov-Smirnov test was used for the normal distribution of all data. All values were expressed as means ± standard deviation (SD). Multi-group comparisons (different donation frequencies, age categories, regions, blood type groups, and BMI) were conducted by one-way ANOVA followed by LSD post hoc test. The effects of gender on serum calcium and total serum protein were accomplished by using two-tailed unpaired Student's *t* -tests. Poisson analysis was used to determine the associations between donation frequencies and serum calcium, donation frequencies and total serum protein, age and serum calcium, age and total serum protein, BMI and serum calcium, BMI and total serum protein. A correlation coefficients of <0.10, 0.10-0.29, 0.30-0.49, and$\geqslant 0.50$ were considered negligible, small, moderate, and large correlation, respectively. A 95% confidence intervals (CI) was used and a p-value <0.05 was considered significant. Furthermore, binary logistic regression analyses were used to investigate the effect of different influencing factors on odds of abnormal serum calcium and total serum protein. The strengths of the relationships were expressed as odds ratios (ORs) with corresponding 95% CIs, and p values were calculated for the corresponding results in the logistic regression. Statistical analysis was performed using SPSS statistics software version 22.0 (SPSS Inc., Chicago, USA).

2.4 Ethics Statement

This study was approved by the Ethics Committee of the Institute of IBT, CAMS&PUMC (No.2021029). Informed consent was obtained from the individual participants according to the Declaration of Helsinki.

3 Results

In the present study, the mean level of serum calcium (n=3966) and total serum protein (n=3969) were 2.3±0.15 mmol/L and 67.75±6.02 g/L, respectively. Approximately 20.55% （815/3966）plasma donors' serum calcium was lower than normal value range (2.20-2.65 mmol/L). The proportion of donors whose total serum protein level was lower than normal value range (65 - 85g / L) was 27.99% (1111/3969).

3.1 Effects of age, gender and blood type on serum calcium

As shown in Fig. 1A, serum calcium level of the 18-29 year old group was higher than other three different age groups (2.35 ± 0.16 mmol/L vs. 2.29 ± 0.15 mmol/L vs. 2.28 ± 0.14 mmol/L vs. 2.30 ± 0.13 mmol/L; all $p < 0.001$). Significant difference was also noted between 40-49 and 50-60 years old groups (2.28 ± 0.14 mmol/L vs. 2.30 ± 0.13 mmol/L; $p = 0.001$). According with these results, the 18-29 years old group had the lowest abnormal rate of serum calcium level (proportion of people whose serum calcium level was lower than normal value range) among the four different age groups (Table 2, 16.6% vs. 21.5% vs. 24.3% vs. 18.6%). A small and negative correlation ($r = -0.137$, $p<0.001$) between serum calcium levels and age was found (Fig. 3A). Furthermore, there was a clear distinction between genders, where serum calcium levels were observably higher in male than in female (2.32 ± 0.15 mmol/L vs. 2.29 ± 0.14 mmol/L; $p < 0.001$) (Fig. 1A). And the abnormal rate of serum calcium level of male was lower than female (Table 2, 19.6% vs. 21.2%). Whereas ABO blood type showed no effect on serum calcium level (Fig. 1A).

3.2 Effects of age, gender and blood type on total serum protein

Similar to the results of serum calcium, among the four different age groups, the 18-29 year old group had the highest total serum protein level (69.32 ± 6.40 g/L vs. 67.48 ± 5.98 g/L vs. 67.30 ± 5.93 g/L vs. 67.36 ± 5.70 g/L; all $p < 0.001$) (Fig.1B). Consistent with these results, the 18-29 year old group had the lowest abnormal rate of total serum protein level (proportion of people whose total serum protein level was lower than normal value range) among the four different age groups (Table 2, 22.6% vs. 29.2% vs. 30.5% vs. 28.1%). Age had a small negative correlation with total serum protein levels ($r = -0.120$, $p < 0.001$) (Fig. 3B). Moreover, ABO blood type showed no effect on total serum protein level, nevertheless total serum protein levels of female were observably higher than that of male (67.95 ± 6.09 g/L vs. 67.56 ± 5.95 g/L; $p=0.037$) (Fig. 1B).

3.3 Effects of donation frequencies, regions and BMI on serum calcium

The level of serum calcium was significantly higher in new donors (donation frequency was 0 times) than in 1-6, 7-11 and 12-27 donation times groups (2.33 ± 0.13 mmol/L vs. 2.27 ± 0.16 mmol/L vs. 2.30 ± 0.15 mmol/L vs. 2.31 ± 0.13 mmol/L; all $p < 0.01$)(Fig.2A). The new donors had lower abnormal rate of serum calcium level than other three groups (Table 2, 14.5% vs. 29.5% vs. 20.4% vs. 18.0%). In the five different regions, serum calcium level of Shandong participants was lower than other four groups (2.20 ± 0.10 mmol/L vs. 2.36 ± 0.10 mmol/L vs. 2.29 ± 0.13 mmol/L vs. 2.27 ± 0.15 mmol/L vs. 2.38 ± 0.10 mmol/L; all $p < 0.001$) (Fig.2A). Consistently, the abnormal rate of serum calcium level of Shandong participants was higher than other four groups (Table 2, 47.0% vs. 3.5% vs. 22.0% vs. 29.0% vs. 2.1%). In addition, serum calcium level of low BMI (≤18.4) group was higher than other three groups (2.37 ± 0.14 mmol/L vs. 2.30 ± 0.14 mmol/L vs. 2.29 ± 0.15 mmol/L vs. 2.29 ± 0.15 mmol/L; all $p < 0.001$) (Fig.2A). No correlation between donation frequencies and serum calcium was found, and the correlation between BMI and serum calcium was negligible ($r= - 0.056$, $p=0.0009$)(Fig.3C).

3.4 Effects of donation frequencies, regions and BMI on total serum protein

The effects of donation frequencies, regions and BMI on total serum protein were similar to those of serum calcium. As Fig.2B showed, the total serum protein level of new donors was the highest one of the four different donation frequencies groups (69.89 ± 5.25 g/L vs. 66.47 ± 6.63 g/L vs. 67.39 ± 6.40 g/L vs. 67.25 ± 5.14 g/L, $p < 0.001$). Shandong participants' total serum protein level was lower than other four regions groups (64.23 ± 7.32 g/L vs.69.53 ± 4.57 g/L vs. 67.31 ± 5.24 g/L vs. 67.03 ± 6.27 g/L vs. 70.57 ± 4.13 g/L; all $p < 0.001$). And total serum protein level of low BMI group was higher than other three groups (69.87 ± 6.08 g/L vs. 67.58 ± 6.10 g/L vs. 67.47 ± 6.12 g/L vs. 67.48 ± 6.10 g/L; $p=0.001$, respectively).

3.5 Results of regression analysis between influencing factors and odds of abnormal serum

calcium/total serum protein

The results of binary logistic regression analysis indicated that donation frequencies, age, BMI and regions were significantly associated with a higher risk of low serum calcium level. The OR of repeat donors (donation frequencies were 1-6 times and 7-11 times) were 3.004 times and 1.610 times higher than that of new donors, respectively. The OR of older participants (aged 30-39 years, 40-49 years, and 50-60 years) were 1.392 times, 2.200 times and 1.748 times higher than that of younger participants (aged 18-29 years). The OR of donors whose BMI was 24.0-27.9 was 1.872 times higher than that of donors whose BMI was less than 18.5. The OR of Jiangxi, Hubei, Sichuan and Ningxia participants were decreased by 72.8% (95% CI: 0.216-0.342, $p<0.001$), 61.4% (95% CI: 0.309-0.482, $p<0.001$), 97.0% (95% CI: 0.020-0.046, $p<0.001$) and 97.8% (95% CI: 0.013-0.036, $p<0.001$) compared to Shandong participants, respectively (Table 3).

In the binary logistic regression model, donation frequencies, gender, age and regions were significant determinants factors of odds of abnormal total serum protein. The abnormal total serum protein odds of donors whose donation frequencies were 1-6 times, 7-11 times and 12-27 times were 3.494 times (95% CI: 2.781-4.390, $p<0.001$), 2.366 times (95% CI: 1.875-2.984, $p<0.001$), and 2.179 times (95% CI: 1.721-2.760, $p<0.001$) higher than that of new donors, respectively. The OR of female was decreased by 17.1% (95% CI: 0.709-0.969, $p = 0.019$) compared to male. Donors aged 30-39 years, 40-49 years, and 50-60 years had 1.436 times (95% CI: 1.115-1.850, $p=0.005$), 1.889 times (95% CI: 1.489-2.398, $p<0.001$), and 1.682 times (95% CI: 1.309-2.163, $p<0.001$) higher abnormal total serum protein odds as compared to donors aged 18-29. The odds of abnormal total serum protein in donors of Jiangxi, Hubei, Sichuan and Ningxia were decreased by 61.7% (95% CI: 0.309-0.474, $p<0.001$), 60.4% (95% CI: 0.319-0.491, $p<0.001$), 88.8% (95% CI: 0.086-0.146, $p<0.001$) and 91.9% (95% CI: 0.061-0.108, $p<0.001$) compared to Shandong participants, respectively (Table 4).

4 Discussion

SP donors provide the starting material for manufacture of PDMPs. In China, SP is collected through plasmapheresis. And plasmapheresis donation is more frequently than whole-blood donation. Detailedly, the interval between two donations shall not be less than 6 months for whole-blood donation and shall not be less than 14 days for plasma donation [12, 13]. Donor safety has been a major concern throughout plasmapheresis donation. In this study, we evaluated the influences of donation frequencies, blood type, gender, age, regions, and BMI on serum calcium and total serum protein in the Chinese plasma donation population.

Citrate was used as anticoagulant during apheresis donations to prevent coagulation and clotting in the apheresis circuit [14]. As citrate is a chelator of ionized calcium, infusing sodium citrate will reduce blood level of ionized calcium[15]. When plasma ionized calcium below 0.9 mmol/L, clinical symptoms of hypocalcemia and hypotension will appear [16]. It was reported that about 1% of first donations and about 0.3% of repeat donations had moderate to severe citrate effects [17]. Furthermore, acute perturbations in calcium metabolism followed apheresis donation [11, 17, 18]. It is well known that serum calcium exists in three forms: ionized, protein-bound, and complexed. Among them, the normal value of ionized calcium is about half of the total serum calcium [19]. Our study showed serum calcium level was lower than normal value range in 20.55%（815/3966）of the plasma donors, which implyed some plasma donors had the risk of low ionic calcium. If these plasma donors continue to donate plasma without treatment, they will be at a higher risk of hypocalcemia. In addition, in the investigation by sweegers *et al*, age was significantly associated with multiple post-donation symptoms in whole blood donors [20]. Similarly, in the present study, there was a negative correlation ($r = -0.137$, $p < 0.001$) between age and serum calcium levels of plasma donors. We also found gender, donation frequencies, regions and BMI had influences on serum calcium level, whereas no significant differences were calculated among different blood type groups. Furthermore, the results of logistics regression analysis showed donation frequencies, age, BMI and regions were significantly associated with a higher risk of low serum calcium level. The repeat donors, coming from Shandong province, with 30-60 years old and the BMI of 24.0-27.9, were more likely to have a lower serum calcium level. These data demonstrated that it is necessary for some plasma donors to supplement calcium in order to maintain health and reduce the possible risk of hypocalcemia. In this process, the effects of age, donation frequency, BMI and region should be taken into account.

Plasmapheresis donation is a process that takes only plasma from the donor while the cellular components are returned. Except water, plasma contains salts, enzymes, antibodies and other proteins. Plasma proteins, such as albumin, immunoglobulins, and coagulation factors, are the main source of PDMPs. Moreover, they are closely related to the immune system and maintain intravascular colloid osmotic pressure, transportation of various metabolites, and regulation of numerous physiological functions [21-24]. It was reported that the loss of plasma proteins are associated with hypoproteinemia, hypoalbuminemia, hypogammaglobulinemia, etc.[25]. In present study, we found the total serum protein levels of 27.99% (1111/3969) donors were lower than normal value range (65 - 85g / L). This result indicated that some plasma donors have the risk of hypoproteinemia. The binary logistic regression analysis results showed, donation frequencies, gender, age and regions were significant determinants factors of the odds of abnormal total serum protein. These results suggest that we should pay more attention to total serum protein level of donors who being repeat donors, being male, aged 30-60 years, and come from Shandong province. Furthermore, we found the total serum protein level of different donation frequencies groups were significant different (except 7-11 group vs. 12-27 group) and the total serum protein level of new donors was higher than repeat donors. R Laub *et al.* found plasma protein levels were significant different among plasmas collected with different techniques and frequencies [26], which were comparable with our results. Moreover, Tian *et al.* observed the total serum protein levels decreased with increasing age in elderly Chinese [27]. Similar to this result, we found age was negative correlated with total serum protein levels ($r = -0.120$, $p < 0.001$) in plasma donors. Moreover, we confirmed that the total serum protein level of plasma donors also been influenced by gender, regions and BMI. These results suggest that some plasma donors have the risk of hypoproteinemia. In order to reduce the risk of hypoproteinemia, attention should be paid to the influencing factors such as donation frequencies, gender, age and regions.

Conclusion

In summary, our study clearly suggested that donation frequency, gender, age, regions, and BMI can influence levels of serum calcium and total serum protein. In plasma donors, older age, being repeat donors, BMI was 24.0-27.9, and come from Shandong province were significantly associated with higher odds of abnormal serum calcium. Furthermore, being repeat donors, older age, being male, and come from Shandong province were significantly correlated with higher odds of abnormal total serum protein. Accordingly, in order to reduce the probability of low serum calcium and low total serum protein, more attention should be paid to the age, donation frequency and region of plasma donors. This study's findings provide new information on a Chinese plasma donors and may contribute toward improving the safety of plasma donation.

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Bin Liu conceived and designed the experiments, performed the experiments, conducted the collection of data, analyzed the data, prepared figures and/or tables, and wrote the paper. Demei Dong performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, and participated in the writing of the paper. Zongkui Wang participated in the analysis of data and in the writing of the report. Yang Gao performed the experiments and participated in the collection of data. Ding Yu conceived and designed the experiments. Shengliang Ye contributed reagents/materials/analysis tools, prepared figures and/or tables. Xi Du performed the experiments, and analyzed the data. Li Ma contributed reagents/materials/analysis tools, performed the experiments. Haijun Cao conceived and designed the experiments, reviewed drafts of the paper. Fengjuan Liu participated in the design of the study, in the analysis of data and in the writing of the report. Rong Zhang participated in the design of the study, analyzed the data, wrote the paper, prepared figures and/or tables, and reviewed drafts of the paper. Changqing Li conceived and designed the experiments, obtained permission from the ethics committee, supervised the study, and reviewed drafts of the paper.

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Conflict of interest

The authors have disclosed no conflicts of interest.

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Tables

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Figure legends

Fig.1 Effects of age, gender and blood type on distribution of serum calcium and total serum protein levels. The dotted line (…) showing the 25th -75th quartiles, and the center lines (- -) representing the median values. The effects of age and blood type were calculated using one-way ANOVA followed by LSD post hoc test, whereas the influence of gender was calculated using two-tailed unpaired Student's t -tests. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (A) Serum calcium; (B) Total serum protein.

Fig.2 Effects of donation frequencies, regions and BMI on distribution of serum calcium and total serum protein levels. The dotted line (…) showing the 25th -75th quartiles, and the center lines (- -) representing the median values. The effects of donation frequencies, regions and BMI were calculated using one-way ANOVA followed by LSD post hoc test. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (A) Serum calcium; (B) Total serum protein.

Fig.3 Associations of serum calcium and age, total serum protein and age, serum calcium and BMI, and total serum protein and BMI. Bivariate correlation analysis was used and the diagonal lines indicate linear regression. (A) Association of serum calcium and age; (B) Association of total serum protein and age; (C) Association of serum calcium and BMI; (D) Association of total serum protein and BMI. Serum calcium and total serum protein were small negative association with age.

Table 1(on next page)

Characteristics of the participants

Table 1. Characteristics of the participants

		serum calcium study (n=3, 966)	total serum protein study (n=3, 969)
Age (years)		40.50±11.53	40.49±11.53
Gender	Male	1,986 (50.1%)	1,989 (50.1%)
	Female	1,980 (49.9%)	1,980 (49.9%)
Blood type	O	1,286 (32.4%)	1,288 (32.5%)
	A	1,224 (30.9%)	1,225 (30.9%)
	B	1,119 (28.2%)	1,119 (28.2%)
	AB	337 (8.5%)	337 (8.5%)
Donation frequencies [a]	0	1,000 (25.2%)	1,000 (25.2%)
	1-6	988 (24.9%)	991 (25.0%)
	7-11	978 (24.7%)	978 (24.6%)
	12 -27	1,000 (25.2%)	1,000 (25.2%)
Regions	Sichuan	799 (20.1%)	799 (20.1%)
	Jiangxi	799 (20.1%)	799 (20.1%)
	Hubei	798 (20.1%)	798 (20.1%)
	Shandong	772 (19.5%)	775 (19.5%)
	Ningxia	798 (20.1%)	798 (20.1%)
BMI (kg/m^2) [b]	＜ 18.5	86 (2.4%)	85 (2.4%)
	18.5-23.9	1,361 (38.2%)	1,363 (38.2%)
	24.0-27.9	1,404 (39.4%)	1,406 (39.4%)
	⩾ 28	716 (20.1%)	716 (20.1%)

BMI: Body mass index. Data are shown as n (%), mean ± standard deviation. [a] number of donors who made a certain number of donations in the year prior to this sample collection; [b] excluding incomplete information and duplicate samples, 3,567 and 3,570 subjects were respectively available to serum calcium and total serum protein research in this study.

Table 2(on next page)

The proportion of people whose detection value is lower than the normal value range in different groups

Table 2. The proportion of people whose detection value is lower than the normal value range in different groups

		serum calcium	total serum protein
Age (years)	18-29	16.6% (131/788)	22.6% (178/789)
	30-39	21.5% (170/791)	29.2% (231/791)
	40-49	24.3% (295/1212)	30.5% (383/1254)
	50-60	18.6% (219/1175)	28.1% (319/1135)
Gender	Male	19.6% (389/1986)	28.8% (573/1989)
	Female	21.2% (426/1980)	27.2% (538/1980)
Blood type	O	21.2% (273/1286)	27.3% (352/1288)
	A	20.2% (247/1224)	27.9% (342/1225)
	B	20.3% (227/1119)	28.6% (320/1119)
	AB	20.2% (68/337)	28.8% (97/337)
Donation frequencies [a]	0	14.5% (145/1000)	16.1% (161/1000)
	1-6	29.5% (291/988)	36.8% (365/991)
	7-11	20.4% (199/978)	29.5% (288/978)
	12 -27	18.0% (180/1000)	29.7% (297/1000)
Regions	Sichuan	3.5% (28/799)	13.6% (109/799)
	Jiangxi	22.0% (176/799)	31.9% (255/799)
	Hubei	29.0% (231/798)	33.7% (269/798)
	Shandong	47.0% (363/772)	52.5% (407/775)
	Ningxia	2.1% (17/798)	8.9% (71/798)
BMI (kg/m^2) [b]	$<$ 18.5	14.0% (12/86)	21.2% (18/85)
	18.5-23.9	21.3% (290/1361)	30.4% (414/1363)
	24.0-27.9	23.3% (327/1404)	29.5% (414/1406)
	$\geqslant$ 28	23.2% (166/716)	28.8% (206/716)

BMI: Body mass index. The normal values of serum calcium and total serum protein were 2.20-2.65 mmol/L and 65-85 g/L respectively. [a] number of donors who made a certain number of donations in the year prior to this sample collection; [b] excluding incomplete information and duplicate samples, 3,567 and 3,570 subjects were respectively available to serum calcium and total serum protein research in this study.

Table 3(on next page)

Binary logistic regression analysis between influencing factors and odds of abnormal serum calcium (lower than normal value range)

Table 3. Binary logistic regression analysis between influencing factors and odds of abnormal serum calcium (lower than normal value range)

	B	SE (B)	Wald χ ²	*p*-value	OR	OR (95% CI)
Donation frequencies						
0	1.00 (referent)					
1-6	1.100	0.127	74.546	0.000 *	3.004	2.340-3.855
7-11	0.476	0.132	13.081	0.000 *	1.610	1.244-2.084
12-27	0.159	0.137	1.358	0.244	1.173	0.897-1.533
Gender						
Male	1.00 (referent)					
Female	0.029	0.092	0.099	0.753	1.029	0.860-1.231
Age (years)						
18-29	1.00 (referent)					
30-39	0.331	0.146	5.103	0.024 *	1.392	1.045-1.855
40-49	0.788	0.138	32.473	0.000 *	2.200	1.677-2.885
50-60	0.558	0.147	14.469	0.000 *	1.748	1.311-2.330
Blood type						
A	1.00 (referent)					
B	- 0.110	0.117	0.885	0.347	0.896	0.713-1.126
AB	- 0.172	0.171	1.015	0.314	0.842	0.602-1.177
O	0.040	0.111	0.132	0.717	1.041	0.837-1.295
BMI						
＜18.5	1.00 (referent)					
18.5-23.9	0.513	0.318	2.597	0.107	1.670	0.895-3.115
24.0-27.9	0.627	0.318	3.901	0.048 *	1.872	1.005-3.489
⩾28	0.621	0.324	3.686	0.055	1.861	0.987-3.509
Regions						
Shandong	1.00 (referent)					
Jiangxi	- 1.304	0.118	122.388	0.000 *	0.272	0.216-0.342
Hubei	- 0.951	0.113	70.327	0.000 *	0.386	0.309-0.482
Sichuan	- 3.501	0.212	271.501	0.000 *	0.030	0.020-0.046
Ningxia	- 3.825	0.258	220.059	0.000 *	0.022	0.013-0.036

The following factors are significantly associated with odds of abnormal serum calcium – donation frequencies, age, and regions.

n = 3,966 in analysis of donation frequencies, gender, age, blood type and regions; n = 3,567 in analysis of BMI.

CI = confidence interval, OR = odds ratios.

∗ $p < 0.05$ by Binary logistic regression analysis.

Table 4(on next page)

Binary logistic regression analysis between influencing factors and odds of abnormal total serum protein (lower than normal value range)

Table 4. Binary logistic regression analysis between influencing factors and odds of abnormal total serum protein (lower than normal value range)

	B	SE (B)	Wald χ ^{2}	*p*-value	OR	OR (95% CI)
Donation frequencies						
0	1.00 (referent)					
1-6	1.251	0.116	115.487	0.000 *	3.494	2.781-4.390
7-11	0.861	0.119	52.795	0.000 *	2.366	1.875-2.984
12-27	0.779	0.120	41.805	0.000 *	2.179	1.721-2.760
Gender						
Male	1.00 (referent)					
Female	- 0.188	0.080	5.546	0.019 *	0.829	0.709-0.969
Age (years)						
18-29	1.00 (referent)					
30-39	0.362	0.129	7.865	0.005 *	1.436	1.115-1.850
40-49	0.636	0.122	27.357	0.000 *	1.889	1.489-2.398
50-60	0.520	0.128	16.499	0.000 *	1.682	1.309-2.163
Blood type						
A	1.00 (referent)					
B	- 0.017	0.101	0.029	0.864	0.983	0.806-1.198
AB	- 0.080	0.148	0.289	0.591	0.924	0.691-1.234
O	- 0.080	0.097	0.669	0.413	0.923	0.763-1.118
BMI						
＜18.5	1.00 (referent)					
18.5-23.9	0.485	0.272	3.178	0.075	1.624	0.953-2.767
24.0-27.9	0.440	0.272	2.625	0.105	1.553	0.912-2.647
⩾28	0.408	0.278	2.151	0.142	1.503	0.872-2.593
Regions						
Shandong	1.00 (referent)					
Jiangxi	- 0.961	0.110	79.916	0.000 *	0.383	0.309-0.474
Hubei	- 0.927	0.110	70.917	0.000 *	0.396	0.319-0.491
Sichuan	- 2.186	0.134	266.733	0.000 *	0.112	0.086-0.146
Ningxia	- 2.516	0.147	294.796	0.000 *	0.081	0.061-0.108

The following factors are significantly associated with odds of abnormal total serum protein – donation frequencies, gender, age, and regions.

n = 3,969 in analysis of donation frequencies, gender, age, blood type and regions; n = 3,570 in analysis of BMI.

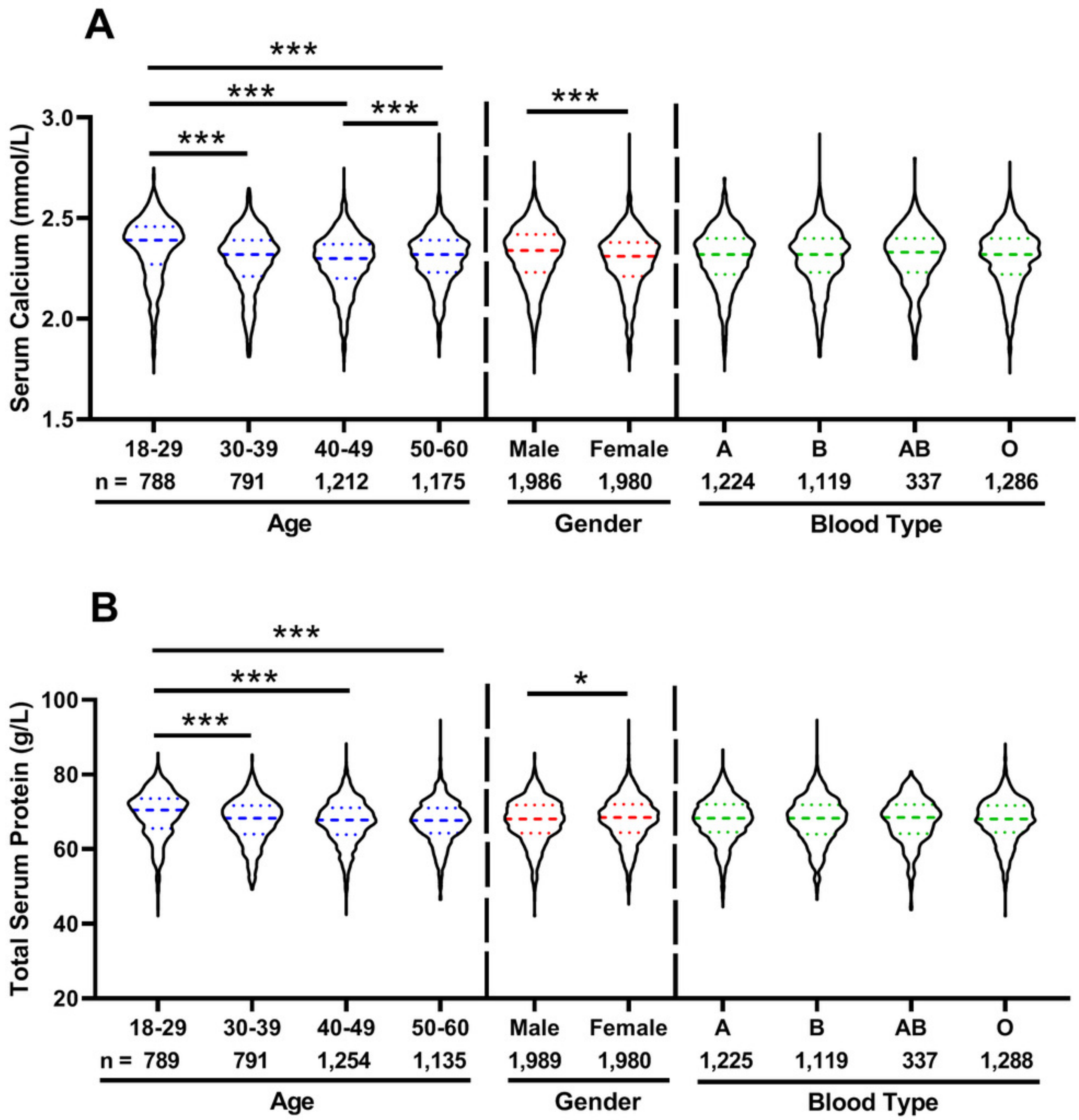
CI = confidence interval, OR = odds ratios.

∗ $p < 0.05$ by Binary logistic regression analysis.

Figure 1

Effects of age, gender and blood type on distribution of serum calcium and total serum protein levels.

The dotted line (…) showing the 25th -75th quartiles, and the center lines (- -) representing the median values. The effects of age and blood type were calculated using one-way ANOVA followed by LSD post hoc test, whereas the influence of gender was calculated using two-tailed unpaired Student's t -tests. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (A) Serum calcium; (B) Total serum protein.


A
***
***
***
***
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Serum Calcium (mmol/L)
3.0
2.5
2.0
1.5
18-29
30-39
40-49
50-60
Male
Female
A
B
AB
O
n = 788
791
1,212
1,175
1,986
1,980
1,224
1,119
337
1,286
Age
Gender
Blood Type
B
***
***
***
*
Total Serum Protein (g/L)
100
80
60
40
20
18-29
30-39
40-49
50-60
Male
Female
A
B
AB
O
n = 789
791
1,254
1,135
1,989
1,980
1,225
1,119
337
1,288
Age
Gender
Blood Type

Figure 2

Effects of donation frequencies, regions and BMI on distribution of serum calcium and total serum protein levels.

The dotted line (…) showing the 25th -75th quartiles, and the center lines (- -) representing the median values. The effects of donation frequencies, regions and BMI were calculated using one-way ANOVA followed by LSD post hoc test. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. (A) Serum calcium; (B) Total serum protein.

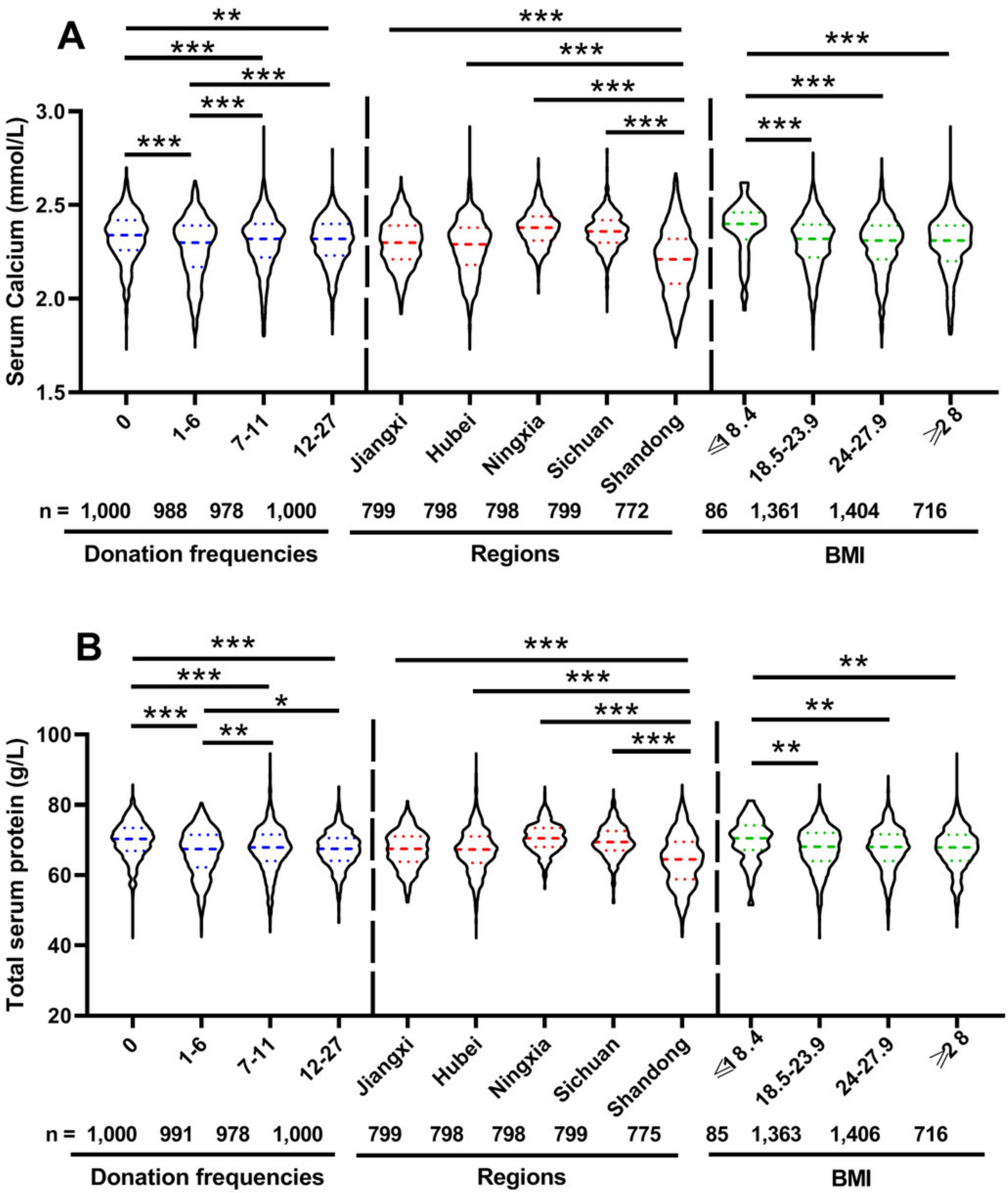
A
Serum Calcium (mmol/L)
0
1-6
7-11
12-27
Jiangxi
Hubei
Ningxia
Sichuan
Shandong
≤18.4
18.5-23.9
24-27.9
≥28
n = 1,000 988 978 1,000 799 798 798 799 772 86 1,361 1,404 716
Donation frequencies
Regions
BMI
B
Total serum protein (g/L)
n = 1,000 991 978 1,000 799 798 798 799 775 85 1,363 1,406 716
Donation frequencies
Regions
BMI

Figure 3

Associations of serum calcium and age, total serum protein and age, serum calcium and BMI, and total serum protein and BMI.

Bivariate correlation analysis was used and the diagonal lines indicate linear regression. (A) Association of serum calcium and age; (B) Association of total serum protein and age; (C) Association of serum calcium and BMI; (D) Association of total serum protein and BMI. Serum calcium and total serum protein were small negative association with age.