

# Elevated Serum Aflatoxin B1 -Lysine and Liver Function Abnormalities Associated with Traditional Home-made Peanut Oil Consumption in Guangzhou

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## Research Article

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## Abstract

## Background

This study assessed serum aflatoxin B<sub>1</sub>-lysine (AFB<sub>1</sub>-Lys) adduct levels and the risk of liver function abnormalities among Guangzhou residents with high or ordinary consumption of traditional homemade peanut oil.

## Methods

From 2022 to 2024, demographic data and serum samples were collected from residents in areas with high and ordinary consumption of traditional homemade peanut oil in Guangzhou, China. Serum AFB<sub>1</sub>-Lys adduct levels were measured by enzyme-linked immunosorbent assay (ELISA). Propensity score matching (PSM) was performed using R version 4.4.3 to improve baseline comparability, followed by binary logistic regression to assess the association between residential region and liver function abnormalities.

## Result

Serum AFB<sub>1</sub>-Lys adduct levels were higher in high-consumption areas than in ordinary-consumption areas ( $4.58 \pm 4.00$  ng/mL). After propensity score matching and covariate adjustment, high-consumption area residence was significantly associated with liver function abnormalities.

## Conclusion

Residents in areas with higher consumption of traditional home-made peanut oil in Guangzhou have significantly elevated serum AFB<sub>1</sub>-Lys levels and an increased risk of liver function abnormalities, suggesting that prolonged AFB<sub>1</sub> exposure may contribute to liver function abnormalities.

## Introduction

Aflatoxins (AFs) were first identified in 1960 and are highly toxic secondary metabolites predominantly synthesized by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Eaton and Gallagher 1994; Abrar et al. 2013). To date, over 20 structurally related aflatoxin compounds have been isolated and characterized, including aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), aflatoxin B<sub>2</sub>, aflatoxin B<sub>2a</sub>, and aflatoxin G<sub>1</sub>. Among these, AFB<sub>1</sub> is the most commonly encountered in the natural environments, exhibiting the highest frequency of detection and the greatest toxicity (Dai et al. 2022; Deng et al. 2025). Extensive research has demonstrated that AFs induce significant hepatotoxic, genotoxic, and immunotoxic effects across a broad spectrum of organisms (Mughal et al. 2017; Chuaysrinule et al. 2023; Martínez et al. 2023; Liu et al. 2023). Consequently, the International Agency for Research on Cancer of the World Health Organization has classified AFs as Group 1 carcinogens (Groopman and Kensler 2005).

AFs primarily contaminate cereals, edible oils, and their derivative products, such as peanuts and peanut oil, maize, rice, and cottonseed, with occasional detection in certain spices and dairy products. The optimal temperature range for the growth of AF-producing fungi is approximately 33–38°C; which explains the predominance of contamination in tropical and subtropical regions (Netto-Ferreira et al. 2011). Furthermore, AFs possess relatively stable physicochemical properties, rendering them resistant to degradation during conventional food processing and cooking procedures. Consequently, dietary intake constitutes the principal exposure pathway for the general population lacking occupational contact with these toxins (Raters and Matissek 2008; Zhang et al. 2020; Pickova et al. 2021).

In recent years, shifts in dietary habits alongside the persistent use of traditional food processing techniques in rural areas have impeded effective control of dietary AFB<sub>1</sub> exposure in China. Guangdong Province, situated in southern China, experiences a warm and humid climate throughout the year, with extended spring and summer seasons. This subtropical environment fosters favorable conditions for the proliferation, reproduction, and toxin production of AF-producing fungi. Additionally, traditional home-made peanut oil, produced via physical pressing methods in family-operated or small-scale oil mills, remains one of the most commonly consumed edible oils among Guangdong residents. The quality and safety of this oil have been longstanding concerns within both societal and academic contexts (Song et al. 2019). Prior studies have indicated that suburban populations in Guangzhou are subject to a measurable degree of dietary AFs exposure attributable to the widespread consumption of traditional home-made peanut oil (Zhang et al. 2020).

Following human ingestion, AFB<sub>1</sub> undergoes primary metabolism and activation in the liver via cytochrome P450 enzymes, notably CYP1A2 and CYP3A4, resulting in the formation of the highly reactive AFB<sub>1</sub>-8,9-epoxide. This metabolite can covalently bind to DNA, producing genotoxic adducts, and can also interact with lysine residues in plasma albumin to generate stable aflatoxin B<sub>1</sub>-lysine adducts (AFB<sub>1</sub>-Lys) (Kensler et al. 2011; Ferreira et al. 2019). Empirical evidence indicates that the half-life of AFB<sub>1</sub>-Lys in human circulation is prolonged, exceeding two months, thereby serving as a biomarker that reflects individual dietary AFB<sub>1</sub> exposure over the preceding 2–3 months (Jager et al. 2016; Zhong et al. 2024). Compared to other biomarkers indicative of short-term exposure, AFB<sub>1</sub>-Lys demonstrates greater stability and has been extensively utilized in population exposure assessments and nutritional epidemiology studies.

The deleterious health effects of AFB<sub>1</sub> are also manifested in its chronic hepatotoxicity (Liu et al. 2014; Cao et al. 2022). Consequently, it is imperative to conduct comprehensive risk assessments of liver function abnormalities among populations residing in areas with varying levels of AFB<sub>1</sub> exposure, grounded in well-characterized exposure data. Given the multifactorial determinants influencing liver function within populations, this study employed propensity score matching (PSM) subsequent to quantifying subjects' AFB<sub>1</sub> exposure, thereby enhancing comparability between two regions through the balancing of key confounding variables.

The present study aimed to quantify and compare internal AFB<sub>1</sub> exposure levels among residents in regions characterized by high versus typical consumption of traditional home-made peanut oil in Guangzhou. Furthermore, it sought to examine the influence of elevated intake of traditional home-made peanut oil on the risk of AFB<sub>1</sub> exposure. Concurrently, PSM was utilized to equilibrate covariate distributions between the two regions, facilitating a subsequent evaluation of the risk of liver function abnormalities among residents in high-consumption areas of traditional home-made peanut oil.

## Materials and Methods

### 2.1 Determination of the Survey Region

Participant selection was conducted based on three dimensions, namely consumption proportion, consumption level, and population comparability. Previous studies reported that the average per capita vegetable oil consumption in Guangzhou was 25.5 g/day (Zhang et al. 2017), which was adopted as the reference threshold for classifying consumption levels. Using dietary survey data and community health records provided by the Guangzhou Center for Disease Control and Prevention (Guangzhou CDC), survey sites were selected through a typical sampling method. Xiao Lou Town in Zengcheng District was identified as a representative high-consumption area for traditional home-made peanut oil, whereas Dashi Town in Panyu District was selected as a representative general-consumption area.

The high-consumption area was defined as a region in which  $\geq 90\%$  of residents had consumed traditional home-made peanut oil for more than one year, with a per capita daily consumption of locally produced peanut oil  $\geq 25.5$  g/day. In contrast, the general-consumption area was defined as a region in which  $\leq 50\%$  of residents had a history of consuming traditional home-made peanut oil for more than one year, and the per capita daily consumption of locally produced peanut

oil was < 25.5 g/day. To ensure comparability between the two study populations, regions with similar age structures and dietary patterns were selected based on community health records and dietary survey data obtained from the Guangzhou CDC.

## 2.2 Sampling Strategy

The study participants were chosen through a two-stage probability sampling procedure. Initially, three villages or neighborhood committees were independently selected from each of the two survey regions using a probability proportional to size sampling technique, whereby the likelihood of selection corresponded to the registered population size of each committee. Subsequently, in the second stage, a random sample of 70 households—comprising approximately 350 individuals—was drawn from each selected committee to constitute the final survey sample.

## 2.3 Subsurface exploration

Between 2022 and 2024, all participants completed a questionnaire survey and underwent a physical examination to gather data on individual characteristics, lifestyle factors, occupational information, and dietary habits. To ensure the sample's representativeness, all participants were long-term local adults (hereafter collectively referred to as residents). Fasting venous blood samples were collected from each participant in the morning to assess levels of alanine aminotransferase, aspartate aminotransferase, and serum AFB<sub>1</sub>-Lys. Written informed consent was obtained from all subjects for their involvement in the questionnaire survey, biological specimen collection, and subsequent data analysis.

## 2.4 Laboratory Examination

### 2.4.1 Materials and Instrumentation

The AFB<sub>1</sub>-Lys ELISA Scientific Research Kit (Jiangsu Jingmei Biotechnology Co., Ltd.) and the iMark microplate reader (Bio-Rad Laboratories) were used in this study.

### 2.4.2 Methodological Procedures

All experimental procedures were performed in strict accordance with the manufacturer's guidelines. Blood samples collected from participants were centrifuged within two hours of collection, and the resulting plasma was aliquoted and stored at -80°C until further analysis. The detection protocol involved the preparation of blank control wells (B<sub>0</sub>, containing 50 µL of 0.00 ng/mL standard), six duplicate standard curve wells with varying concentrations (0.10, 0.30, 0.90, 2.70, and 8.10 ng/mL), and sample wells containing 50 µL of serum diluted eightfold (prepared by mixing 6.25 µL of serum with 43.75 µL of diluent) on a microplate. Subsequently, 50 µL of AFB<sub>1</sub>-Lys enzyme-labeled antibody was added to each well, followed by incubation at 37.0°C for 30 minutes. After incubation, the contents were discarded, and the plate was washed five times with wash buffer and blotted dry. Chromogenic solutions A and B (50 µL each) were then added sequentially, and after 10 minutes of color development at 37.0°C in the dark, 50 µL of stop solution was introduced. Absorbance was immediately measured at 450 nm using a microplate reader. Sample concentrations were determined by fitting the standard curve (with R<sup>2</sup> ≥ 0.99) using a four-parameter logistic regression model and were adjusted to account for the eightfold dilution factor.

### 2.4.3 Quality Assurance and Control

The ELISA kit exhibited a limit of detection of 0.05 ng/mL and a limit of quantification of 0.10 ng/mL, with a linear detection range spanning from 0.10 to 8.10 ng/mL. Throughout the assay, the absorbance values of both samples and standards were normalized against the blank control to compute percentage absorbance. Standard curves were generated using ELISA Calc software (version 0.1), achieving a coefficient of determination of 0.99984. Sample absorbance readings were interpolated on the standard curve to ascertain concentrations, which were subsequently adjusted by an eightfold dilution factor to obtain the actual analyte concentrations. Method validation revealed intra-assay coefficients of variation

between 3.26% and 13.52%, alongside an inter-assay coefficient of variation of 5.19%, thereby confirming the assay's precision and reliability.

## 2.5 Propensity Score Match

The liver serves as the principal site for the metabolic activation of AFB<sub>1</sub> and represents a critical target organ for its toxic effects. To further evaluate the health status of residents across regions with varying levels of traditional home-made peanut oil consumption, this study utilized PSM to pair individuals from areas with ordinary consumption to those from high-consumption areas. Subsequently, the study examined differences in the risk of liver function abnormalities between these groups. Liver function abnormality was defined based on the reference intervals for commonly employed clinical biochemical assays—specifically serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and  $\gamma$ -glutamyltransferase—outlined in Part 1 of the WS/T 404.1–2012 guidelines issued by the Ministry of Health of the People's Republic of China. An abnormal liver function was characterized by any elevation of ALT or AST exceeding the upper limit of normal, with thresholds set at ALT  $\geq$  50 U/L or AST  $\geq$  40 U/L for males, and ALT  $\geq$  40 U/L or AST  $\geq$  35 U/L for females (Ministry of Health, People's Republic of China 2012).

Given that hepatitis B virus infection significantly impairs hepatic function and that the extent of liver damage differs according to the stage of the disease, coupled with the possibility that various medications (such as antibiotics and statins) may provoke drug-induced liver injury, individuals diagnosed with hepatitis B and those who had administered drugs potentially causing hepatic injury within the preceding three months were excluded before PSM. The catalog of medications linked to drug-induced liver injury was verified through a specialized professional database ([CSL STYLE ERROR: reference with no printed form.]).

Drawing upon prior research concerning liver function abnormalities (Cheng et al. 2022; Liang et al. 2025), ten principal confounding variables were identified for PSM. These variables encompassed age, sex, educational attainment, per capita annual household income, body mass index (BMI), alcohol consumption, smoking status, and the presence of hypertension, diabetes, and fatty liver disease. BMI classifications adhered to the 2024 edition of the Guiding Principles for Weight Management issued by the National Health Commission of China, delineated as underweight (BMI < 18.5 kg/m<sup>2</sup>), normal weight (18.5  $\leq$  BMI < 24 kg/m<sup>2</sup>), overweight (24  $\leq$  BMI < 28 kg/m<sup>2</sup>), and obesity (BMI  $\geq$  28 kg/m<sup>2</sup>).

PSM was performed utilizing R software (version 4.4.3) with a 1:1 caliper matching approach, applying a caliper width of 0.02. Statistical analyses were conducted using the “tableone” and “MatchIt” packages. Following the exclusion of individuals with hepatitis B and those with recent exposure to medications potentially influencing liver function, a total of 1,484 participants (942 from Xiao Lou and 542 from Dashi) were eligible for the matching process. Ultimately, 285 participants from Xiao Lou were successfully matched with 285 participants from Dashi.

## 2.6 Statistical Analysis

Data were processed using IBM SPSS Statistics version 27. The Mann-Whitney U test was utilized to compare internal AFB<sub>1</sub> exposure levels between the two regions. Graphical representations were created with Origin 2024. Following PSM, binary logistic regression was conducted to evaluate the risk of liver function abnormalities among residents from the two regions, with a significance threshold set at  $P < 0.05$ . The standard curve for the AFB<sub>1</sub>-lysine ELISA assay was generated using ELISA Calc software (version 0.1). All PSM procedures were carried out in R (version 4.4.3) employing the “tableone” and “MatchIt” packages.

## Results

A total of 2,095 valid questionnaires were obtained, comprising 1,101 from Dashi and 994 from Xiao Lou. The survey findings revealed that the average age of residents in Xiao Lou was 61.12 years, with a gender distribution of 485 males and 509 females. In contrast, residents of Dashi had a mean age of 57.87 years, exhibiting a similar gender distribution

(485 males and 509 females). Furthermore, 91.65% (911 out of 994) of Xiao Lou residents reported consuming traditional home-made peanut oil within the past year, with more than one year of prior consumption experience, compared to 43.51% (479 out of 1,101) of residents in Dashi. The demographic characteristics of the study population from 2022 to 2024 are detailed in Table 1.

Table 1  
Basic Information of Research Subjects from 2022 to 2024

| Years     | Area     | sample size | Age                       | Gender      |
|-----------|----------|-------------|---------------------------|-------------|
|           |          |             | Mean ± standard deviation | Male/female |
| 2022      | Xiao Lou | 325         | 59.79 ± 14.66             | 154/171     |
|           | Da Shi   | 385         | 59.11 ± 14.04             | 181/204     |
| 2023      | Xiao Lou | 329         | 57.87 ± 15.27             | 157/172     |
|           | Da Shi   | 362         | 53.72 ± 12.99             | 122/240     |
| 2024      | Xiao Lou | 340         | 65.55 ± 10.83             | 174/166     |
|           | Da Shi   | 354         | 58.23 ± 14.14             | 122/232     |
| 2022–2024 | Xiao Lou | 994         | 61.12 ± 14.07             | 485/509     |
|           | Da Shi   | 1101        | 57.87 ± 13.60             | 425/676     |
|           | Total    | 2095        | 59.42 ± 13.92             | 910/1185    |

Table 2 presents the concentrations of AFB<sub>1</sub>-Lys adducts in the serum of individuals residing in two distinct regions from 2022 to 2024. The data, gathered over this three-year period, consistently demonstrate that serum AFB<sub>1</sub>-Lys levels in residents of Xiao Lou were significantly elevated compared to those observed in residents of Dashi.

Table 2  
Serum AFB<sub>1</sub> Adduct Levels in Residents of Two Regions (2022–2024) (ng/mL)

| Years     | Area     | sample size | Mean ± standard deviation | Median | Z       | p      |
|-----------|----------|-------------|---------------------------|--------|---------|--------|
| 2022      | Xiao Lou | 325         | 4.21 ± 2.94               | 3.56   | -9.899  | < 0.01 |
|           | Da Shi   | 385         | 2.81 ± 2.92               | 2.47   |         |        |
| 2023      | Xiao Lou | 329         | 5.29 ± 3.30               | 4.77   | -13.424 | < 0.01 |
|           | Da Shi   | 362         | 2.57 ± 1.98               | 2.24   |         |        |
| 2024      | Xiao Lou | 340         | 4.26 ± 5.24               | 1.95   | -3.467  | < 0.01 |
|           | Da Shi   | 354         | 3.96 ± 6.72               | 0.86   |         |        |
| 2022–2024 | Xiao Lou | 994         | 4.58 ± 4.00               | 3.76   | -13.245 | < 0.01 |
|           | Da Shi   | 1101        | 3.10 ± 4.37               | 2.12   |         |        |

Note: Wilcoxon signed rank test was used to compare the serum AFB<sub>1</sub> adduct levels of residents in the two regions. A negative Z value indicates that the level in Xiao Lou is higher than that in Dashi. P value is the result of bilateral test.

Figure 1 displays grouped violin plots that consolidate data collected from 2022 to 2024. The analysis reveals that serum AFB<sub>1</sub>-Lys concentrations among residents in both regions deviate from a normal distribution, exhibiting a pronounced right-skewed pattern. This skewness suggests the existence of a subgroup of individuals with notably elevated exposure

levels. Regarding distributional characteristics, serum AFB<sub>1</sub>-Lys concentrations in residents of Xiao Lou were generally higher compared to those in Dashi, as evidenced by an upward shift in both the median and interquartile range, indicating a higher overall internal exposure in Xiao Lou. Additionally, the distribution of serum AFB<sub>1</sub>-Lys levels in Dashi residents was relatively broad and flattened, whereas the distribution in Xiao Lou was more concentrated. This pattern implies greater inter-individual variability in exposure within Dashi, with a subset of residents experiencing particularly high exposure levels.

Table 3 presents a summary of the confounding variables among residents of Dashi and Xiao Lou before and after propensity score matching. Prior to matching, the sample from Dashi comprised 542 individuals, including 209 males and 333 females, whereas the Xiao Lou cohort consisted of 942 individuals, with 466 males and 476 females. A statistically significant difference in gender distribution was observed between the two areas ( $p < 0.001$ ). Following matching, the mean age of participants was 64.83 years in Dashi and 63.69 years in Xiao Lou. The proportion of individuals reporting a household annual income below 4,000 yuan was 59% in Xiao Lou compared to 30.4% in Dashi. BMI classifications among matched subjects predominantly fell within the normal or overweight categories, representing 82.3% in Xiao Lou and 80.6% in Dashi. Furthermore, over 70% of matched participants in both regions indicated no history of smoking or alcohol consumption. Statistically significant differences between the two regions were identified in terms of gender distribution, educational attainment, household income, and smoking status ( $p < 0.001$ ), whereas no significant differences were found regarding age, BMI, or alcohol consumption ( $p > 0.05$ ).

Table 3

Comparison of Confounding Characteristics Before and After Propensity Score Adjustment in Dashi and Xiao Lou Areas

| Variable                              | Before propensity score adjustment |                    |          | After propensity score weighting |                    |          |         |
|---------------------------------------|------------------------------------|--------------------|----------|----------------------------------|--------------------|----------|---------|
|                                       | Da Shi(n = 542)                    | Xiao Lou (n = 942) | <i>p</i> | Da Shi (n = 285)                 | Xiao Lou (n = 285) | <i>p</i> | SMD     |
| Gender                                |                                    |                    |          |                                  |                    |          |         |
| male                                  | 209 (38.6%)                        | 466 (49.5%)        | < 0.001  | 122 (42.8%)                      | 122 (42.8%)        | 1.000    | < 0.001 |
| female                                | 333 (61.4%)                        | 476 (50.5%)        |          | 163 (57.2%)                      | 163 (57.2%)        |          |         |
| Age (mean (standard deviation))       | 64.83 (9.32)                       | 63.69 (11.67)      | 0.052    | 64.34 (9.35)                     | 64.39 (11.61)      | 0.962    | 0.004   |
| Educational level                     |                                    |                    |          |                                  |                    |          |         |
| No educational qualifications         | 46 (8.5%)                          | 93 (9.9%)          | < 0.001  | 29 (10.2%)                       | 36 (12.6%)         | 0.567    | 0.119   |
| Primary school                        | 18.4 (33.9%)                       | 428 (45.4%)        |          | 107 (37.5%)                      | 105 (36.8%)        |          |         |
| Middle School                         | 169 (31.2%)                        | 281 (29.8%)        |          | 89 (31.2%)                       | 77 (27.0%)         |          |         |
| High school level and above           | 143 (26.4%)                        | 140 (14.9%)        |          | 60 (21.1%)                       | 67 (23.5%)         |          |         |
| Annual per capita family income       |                                    |                    |          |                                  |                    |          |         |
| Less than 4000 yuan                   | 165 (30.4%)                        | 556 (59.0%)        | < 0.001  | 111 (38.9%)                      | 110 (38.6%)        | 0.821    | 0.080   |
| 4000–8000 yuan                        | 122 (22.5%)                        | 168 (17.8%)        |          | 74 (26.0%)                       | 69 (24.2%)         |          |         |
| 8000–12000 yuan                       | 68 (12.5%)                         | 108 (11.5%)        |          | 39 (13.7%)                       | 36 (12.6%)         |          |         |
| More than 12000 yuan                  | 187 (34.5%)                        | 110 (11.7%)        |          | 61 (21.4%)                       | 70 (24.6%)         |          |         |
| BMI                                   |                                    |                    |          |                                  |                    |          |         |
| Underweight                           | 24 (4.4%)                          | 42 (4.5%)          | 0.071    | 15 (5.3%)                        | 12 (4.2%)          | 0.658    | 0.106   |
| Normal weight                         | 207 (38.2%)                        | 425 (45.1%)        |          | 120 (42.1%)                      | 116 (40.7%)        |          |         |
| Overweight                            | 230 (42.4%)                        | 350 (37.2%)        |          | 109 (38.2%)                      | 122 (42.8%)        |          |         |
| Obesity                               | 81 (14.9%)                         | 125 (13.3%)        |          | 41 (14.4%)                       | 35 (12.3%)         |          |         |
| Has a drinking habits or not          |                                    |                    |          |                                  |                    |          |         |
| No                                    | 455 (83.9%)                        | 791 (84.0%)        | 0.424    | 236 (82.8%)                      | 239 (83.9%)        | 0.403    | 0.030   |
| Has been alcohol-free for over a year | 10 (1.8%)                          | 27 (2.9%)          |          | 4 (1.4%)                         | 1 (0.4%)           |          |         |
| Yes                                   | 77 (14.2%)                         | 124 (13.2%)        |          | 45 (15.8%)                       | 45 (15.8%)         |          |         |

| Variable                            | Before propensity score adjustment |                    |          | After propensity score weighting |                    |          |         |
|-------------------------------------|------------------------------------|--------------------|----------|----------------------------------|--------------------|----------|---------|
|                                     | Da Shi(n = 542)                    | Xiao Lou (n = 942) | <i>p</i> | Da Shi (n = 285)                 | Xiao Lou (n = 285) | <i>p</i> | SMD     |
| Has a smoking habit or not          |                                    |                    |          |                                  |                    |          |         |
| No                                  | 447 (82.5%)                        | 670 (71.1%)        | < 0.001  | 222 (77.9%)                      | 221 (77.5%)        | 0.940    | 0.113   |
| Has been smoke-free for over a year | 32 (5.9%)                          | 47 (5.0%)          |          | 17 (6.0%)                        | 19 (6.7%)          |          |         |
| Yes                                 | 63 (11.6%)                         | 225 (23.9%)        |          | 46 (16.1%)                       | 45 (15.8%)         |          |         |
| Has hypertension or not             |                                    |                    |          |                                  |                    |          |         |
| No                                  | 211 (38.9%)                        | 656 (69.6%)        | < 0.001  | 143 (50.2%)                      | 129 (45.3%)        | 0.276    | 0.098   |
| Yes                                 | 331 (61.1%)                        | 286 (30.4%)        |          | 142 (49.8%)                      | 156 (54.7%)        |          |         |
| Has diabetes or not                 |                                    |                    |          |                                  |                    |          |         |
| No                                  | 407 (75.1%)                        | 844 (89.6%)        | < 0.001  | 225 (78.9%)                      | 225 (78.9%)        | 1.0      | < 0.001 |
| Yes                                 | 135 (24.9%)                        | 98 (10.4%)         |          | 60 (21.1%)                       | 60 (21.1%)         |          |         |
| Has fatty liver or not              |                                    |                    |          |                                  |                    |          |         |
| No                                  | 342 (63.1%)                        | 906 (96.2%)        | < 0.001  | 238 (83.5%)                      | 251 (88.1%)        | 0.150    | 0.131   |
| Yes                                 | 200 (36.9%)                        | 36 ( 3.8%)         |          | 47 (16.5%)                       | 34 (11.9%)         |          |         |

Concerning disease prevalence among matched participants in Dashi, 211 individuals (38.9%) were diagnosed with hypertension, 407 (75.1%) with diabetes, and 342 (63.1%) with fatty liver. In contrast, the corresponding figures in Xiao Lou were 656 (69.6%), 844 (89.6%), and 906 (96.2%), respectively. The differences in the prevalence rates of hypertension, diabetes, and fatty liver between these two areas were statistically significant ( $p < 0.001$ ).

Prior to PSM, no significant differences were observed between the two regions in terms of age, BMI, and alcohol consumption. However, other confounding variables and indicators of liver function abnormalities showed marked disparities, suggesting limited comparability regarding liver function outcomes. Consequently, PSM was implemented to ensure a more balanced distribution of confounding factors between the groups.

A matched cohort comprising 285 resident pairs was constructed using a 1:1 matching ratio. Following matching, t-tests and chi-square tests were conducted to assess the balance of covariates between the two regions. Additionally, the Standardized Mean Difference (SMD) for each covariate was computed to evaluate the effectiveness of the matching process, with an SMD threshold of less than 10% considered indicative of satisfactory balance (Caliendo and Kopeinig 2008; Nie et al. 2020). The analysis revealed that post-matching, no significant differences remained in covariates such as age, gender, educational attainment, household per capita annual income, BMI, alcohol consumption status, smoking status, hypertension, diabetes, and fatty liver disease ( $p > 0.05$ ). However, SMD values for alcohol consumption status, educational level, BMI, and fatty liver exceeded the 10% threshold, while SMDs for the other variables were below this cutoff. Therefore, covariates exhibiting SMDs greater than 10% were incorporated into subsequent binary logistic regression models to control for residual confounding effects.

Following PSM, liver function abnormality status among residents was designated as the dependent variable (yes = 1, no = 0). A binary logistic regression model was then developed, with geographic region serving as the primary independent variable. Covariates that remained unbalanced post-matching—including alcohol consumption status, educational attainment, BMI, and presence of fatty liver disease—were concurrently controlled for within the model. The findings from the logistic regression analysis are detailed in Tables 4. The results indicate that, after adjusting for potential confounders, residents of Xiao Lou exhibited a significantly elevated risk of liver function abnormalities relative to those residing in Dashi (OR = 2.741, 95% CI: 1.318–5.699,  $p < 0.01$ ).

Table 4  
Results of logistic regression analysis after propensity score matching

| Variable                              | B       | SE        | $\chi^2$ | $p$   | OR (95% CI)           |
|---------------------------------------|---------|-----------|----------|-------|-----------------------|
| Area                                  |         |           |          |       |                       |
| Dashi*                                |         |           |          |       |                       |
| Xiao Lou                              | 1.008   | 0.373     | 7.287    | 0.007 | 2.741 (1.318, 5.699)  |
| Has a drinking habits or not          |         |           |          |       |                       |
| No*                                   |         |           |          |       |                       |
| Has been alcohol-free for over a year | -18.259 | 17476.793 | 0        | 0.999 | 0                     |
| Yes                                   | 0.475   | 0.416     | 1.305    | 0.253 | 1.608 (0.712, 3.634)  |
| Educational level                     |         |           |          |       |                       |
| No educational qualifications*        |         |           |          |       |                       |
| Primary school                        | 1.07    | 0.766     | 1.95     | 0.163 | 2.916 (0.649, 13.095) |
| Middle School                         | 0.661   | 0.804     | 0.675    | 0.411 | 1.936 (0.4, 9.364)    |
| High school level and above           | 0.932   | 0.802     | 1.35     | 0.245 | 2.539 (0.527, 12.226) |
| BMI                                   |         |           |          |       |                       |
| Underweight*                          |         |           |          |       |                       |
| Normal weight                         | -0.876  | 0.694     | 1.593    | 0.207 | 0.417 (0.107, 1.623)  |
| Overweight                            | -0.77   | 0.693     | 1.232    | 0.267 | 0.463 (0.119, 1.803)  |
| Obesity                               | -0.315  | 0.756     | 0.174    | 0.676 | 0.73 (0.166, 3.209)   |
| Has fatty liver or not                |         |           |          |       |                       |
| No*                                   |         |           |          |       |                       |
| Yes                                   | 0.586   | 0.439     | 1.782    | 0.182 | 1.797 (0.76, 4.248)   |
| Note: *: Control group.               |         |           |          |       |                       |

Moreover, in the multivariable logistic regression model after PSM, serum AFB<sub>1</sub>-Lys levels were included as an independent variable in place of the regional variable. However, serum AFB<sub>1</sub>-Lys levels were not independently associated with abnormal liver function after adjustment for potential confounding factors. To further investigate whether regional differences persisted in internal exposure levels, paired comparisons of serum AFB<sub>1</sub>-Lys concentrations between residents from Xiao Lou and Dashi were conducted following matching. The Wilcoxon paired signed-rank test demonstrated that

serum AFB<sub>1</sub>-Lys levels among residents in Xiao Lou were significantly higher than those among residents in Dashi [3.38 (1.60–5.04) ng/mL vs. 2.15 (0.95–3.45) ng/mL,  $p < 0.001$ ], indicating that residents in the high-consumption area remained exposed to higher levels of AFB<sub>1</sub> despite balancing baseline characteristics through PSM. The negative Z statistic further suggested that serum AFB<sub>1</sub>-Lys concentrations in Xiao Lou exceeded those observed in Dashi.

Although the ELISA assay for serum AFB<sub>1</sub>-Lys in this study was executed in strict adherence to the manufacturer’s instructions, a thorough in-house methodological validation was not undertaken. This validation would ideally encompass the evaluation of measurement uncertainty, recovery experiments, and a systematic assessment of analytical precision. The absence of these validation steps may compromise the reliability of the findings and the reproducibility of the assay. Specifically, the measured concentrations could be influenced by systematic biases of undetermined direction, such as recovery deviations attributable to matrix effects, and the quantitative accuracy at low concentration levels may be limited.

Discussion

4.1 Analysis of Serum AFB<sub>1</sub>-Lys Exposure Levels of Residents in Two Regions

The findings of this investigation revealed that, over a three-year duration, serum AFB<sub>1</sub>-Lys concentrations among inhabitants of Xiao Lou were significantly elevated compared to those in Dashi ( $p < 0.01$ ). Considering that AFB<sub>1</sub>-Lys serves as a biomarker characterized by a relatively prolonged half-life and the capacity to indicate cumulative exposure over time, the consistently higher levels detected in Xiao Lou residents imply sustained and greater internal exposure to AFB<sub>1</sub> within this population.

Table 5 provides a summary of reported internal exposure biomarker levels of AFB<sub>1</sub> across various countries and regions. When compared to prior research, the serum AFB<sub>1</sub>-Lys concentrations identified in residents from both study locations in Guangzhou exceeded those documented in populations from India and Mexico.

Table 5  
Serum AFB<sub>1</sub>-Lys Exposure Levels in Residents of Other Regions

| Area   | Research population    | AFB <sub>1</sub> -Lys level             | Testing method | AFB <sub>1</sub> -Lys unit | References                     |
|--------|------------------------|-----------------------------------------|----------------|----------------------------|--------------------------------|
| India  | Healthy individuals    | 0.3 ± 1.1 (Mean ± Standard Deviation)   | ELISA          | ng/mL                      | (Shukla et al. 2025)           |
| Mexico | Adult                  | 0.172 (Median)                          | LC-MS/MS       | ng/mL                      | (Monge et al. 2023)            |
| Gambia | Pregnant women         | 3.9-458.4 (Detection range)             | ELISA          | pg/mg of albumin           | (Hernandez-Vargas et al. 2015) |
| Malawi | The general population | 20.5 ± 23.4 (Mean ± Standard Deviation) | ELISA          | pg/mg of albumin           | (Seetha et al. 2018)           |

Nonetheless, it should be noted that substantial heterogeneity exists across studies with respect to the types of biomarkers measured, analytical methodologies employed, and units used for reporting results (e.g., ng/mL, pg/mL, or pg/mg albumin), with most earlier studies expressing AFB<sub>1</sub>-Lys levels as pg/mg albumin. Therefore, direct cross-regional comparisons between the current results and those from other studies should be approached with caution due to these methodological and reporting discrepancies.

A comparative analysis of serum AFB<sub>1</sub>-Lys level distributions between the two regions revealed a pronounced right-skewed pattern in the Dashi, suggesting the existence of a subgroup of residents experiencing relatively elevated internal exposure.

This extended tail distribution may be attributed to increased consumption of traditional home-made peanut oil among certain individuals in this locality or to specific occupational exposure factors. Further research is necessary to identify the primary sources of exposure and to establish an evidence base that can inform targeted risk management strategies and policy development.

Moreover, the observed regional disparities in internal exposure levels highlight the critical role of biomonitoring. In contrast to dietary exposure assessments that rely solely on AFB<sub>1</sub> concentrations in food, serum AFB<sub>1</sub>-Lys provides a more direct and comprehensive measure of the actual internal exposure burden at the individual level.

## **4.2 Analysis of the Differences in the Risk of liver function abnormalities between the Two Regions**

This study employed PSM to effectively balance differences in demographic characteristics, lifestyle factors, and selected disease conditions between residents in the high-consumption area of traditional home-made peanut oil (Xiao Lou) and those in the ordinary-consumption area (Dashi). This methodological approach ensured adequate covariate balance between the two groups, thereby establishing a robust foundation for causal inference in assessing the influence of regional factors on the risk of liver function abnormalities.

Following the matching procedure, no statistically significant differences were detected in demographic variables, smoking and alcohol consumption behaviors, or the prevalence of multiple chronic diseases among the 285 matched pairs. This outcome indicates that satisfactory comparability between groups was achieved and that selection bias was substantially mitigated.

In the PSM cohort, covariates exhibiting a SMD exceeding 10% post-matching were incorporated into a binary logistic regression model for further adjustment. The analysis revealed a significant association between regional factors and the incidence of liver function abnormalities. Specifically, individuals residing in Xiao Lou demonstrated a markedly higher risk of liver function impairment compared to those living in Dashi. This finding suggests that residing in areas characterized by high consumption of traditional home-made peanut oil may represent an independent risk factor for hepatic dysfunction. This observation aligns with prior findings regarding serum AFB<sub>1</sub>-Lys adduct exposure levels, wherein residents of Xiao Lou exhibited elevated internal AFB<sub>1</sub> exposure relative to those in Dashi.

Within the binary logistic regression framework, adjustments for drinking status, educational attainment, BMI, and fatty liver status revealed no statistically significant associations between these covariates and liver function abnormalities ( $p > 0.05$ ). Notably, the category “Has been alcohol-free for over a year” displayed a relatively large regression coefficient and standard error, with an odds ratio approaching zero. This instability likely stems from the limited sample size within this subgroup ( $n = 5$  post-matching), resulting in unreliable parameter estimates. Such issues are common in PSM-based analyses and warrant cautious interpretation. Nonetheless, inclusion of this variable in the model aids in reducing potential residual confounding when estimating regional effects.

Furthermore, educational level, BMI, and fatty liver status demonstrated fluctuating odds ratios with wide confidence intervals encompassing unity, suggesting possible but statistically inconclusive associations with liver function abnormalities. Given the current sample size, statistical power may be insufficient to detect moderate effects. Future studies may consider moderately increasing the caliper width in PSM to expand the matched sample size, thereby facilitating more robust evaluation of the potential modifying or interactive roles of these factors in the relationship between AFB<sub>1</sub> exposure and liver dysfunction.

The Wilcoxon paired signed-rank test comparing serum AFB<sub>1</sub>-Lys concentrations between the two regions post-matching demonstrated that levels remained significantly elevated in Xiao Lou relative to Dashi ( $Z = -4.851$  and  $-4.545$ , both  $p < 0.001$ ). Despite effective balancing of demographic characteristics, lifestyle factors, and disease-related confounders via

PSM, substantial regional disparities in AFB<sub>1</sub>-Lys levels persisted. Taken together with the post-matching logistic regression outcomes, these results suggest that the influence of regional factors on liver function abnormalities may be partially mediated by increased internal AFB<sub>1</sub> exposure. Although serum AFB<sub>1</sub>-Lys was not independently associated with liver function abnormalities in the multivariable model, its sustained regional disparity within the matched cohort supports its potential role as a mediator in the pathway linking residential region to hepatic dysfunction, rather than serving as an independent risk factor.

## 4.3 Health Prevention and Control Recommendations

Throughout the production and storage stages, traditional home-made peanut oil is susceptible to contamination by aflatoxins. In recent years, local regulatory agencies have intensified oversight of the production facilities for traditional home-made peanut oil. Between 2015 and 2017, analyses of traditional home-made peanut oil samples from Guangzhou revealed an average AFB<sub>1</sub> contamination level of  $38.74 \pm 47.45$  µg/kg, significantly exceeding the contamination levels observed in commercially available vegetable oils (Zhang et al. 2020). Between 2022 and 2024, analyses performed by the Guangzhou Center for Disease Control and Prevention on commercially available traditional home-made peanut oil demonstrated a reduction in AFB<sub>1</sub> contamination levels to  $3.87 \pm 10.61$  µg/kg. These findings suggest that Guangzhou has attained significant progress in the regulatory oversight of the production and processing of traditional home-made peanut oil.

Due to the climatic conditions in Guangdong, which promote the growth of peanut mold, the level of AFB<sub>1</sub> contamination in traditional home-made peanut oil remains elevated compared to that in commercial vegetable oils during the same timeframe ( $0.61 \pm 1.06$  µg/kg). Furthermore, after employing propensity score matching and controlling for various confounding variables, this study demonstrated that individuals residing in regions with high consumption of traditional home-made peanut oil exhibited a significantly increased risk of liver dysfunction relative to those in areas with average consumption. These results indicate that, despite a marked reduction in AFB<sub>1</sub> contamination levels, prolonged and continuous exposure to traditional home-made peanut oil may still exert detrimental effects on liver function.

Traditional home-made peanut oil not only represents a distinctive aspect of Guangdong's regional culinary heritage but also constitutes a vital source of income for small-scale family-operated workshops. In the current regulatory environment, which has substantially decreased the levels of AFB<sub>1</sub> contamination in traditional home-made peanut oil, risk management strategies should focus on further minimizing health risks to the local population while maintaining the product's regional characteristics. Primarily, the health risks associated with traditional home-made peanut oil stem from the high susceptibility of raw peanuts to mold contamination and the absence of effective detoxification procedures during processing. Consequently, critical control points within the production chain must be identified and managed. Potential interventions include enhancing the grading, screening, and storage protocols for raw peanuts; standardizing pressing and storage conditions in small-scale workshops; and implementing routine inspections alongside risk-based management approaches to prevent highly contaminated products from reaching consumers. Additionally, public health education initiatives can encourage residents to consume traditional home-made peanut oil in a more rational manner, such as by reducing consumption frequency or alternating its use with commercially produced vegetable oils. This strategy, which preserves traditional dietary practices, is likely to be more acceptable and sustainable within the community.

In summary, the risk management of traditional home-made peanut oil should, while acknowledging local dietary practices, implement a comprehensive strategy encompassing source control, process monitoring, and consumer education. This integrated approach has the potential to further mitigate population-wide exposure to AFB<sub>1</sub> and facilitate the standardized advancement of traditional food products within the context of contemporary food safety standards.

This study investigated serum AFB<sub>1</sub>-Lys levels among residents across various consumption regions within Guangzhou City. The findings demonstrated that individuals residing in high-consumption areas exhibited significantly elevated serum

AFB<sub>1</sub>-Lys concentrations compared to those in regions characterized by ordinary consumption, suggesting a greater cumulative exposure burden. Additionally, the study utilized a methodological approach combining propensity score matching with binary logistic regression to rigorously assess the influence of regional disparities on the risk of liver function impairment. After adjusting for demographic variables, pre-existing health conditions, and other key confounders, residents of Xiao Lou were found to have a markedly increased risk of liver function impairment relative to residents of Dashi. These results imply that inhabitants of Guangzhou may be subject to specific public health risks, with high intake of traditional home-made peanut oil potentially contributing to elevated AFB<sub>1</sub> exposure within the body.

## Abbreviations

| Abbreviations         | Full term                         |
|-----------------------|-----------------------------------|
| AFs                   | Aflatoxins                        |
| AFB <sub>1</sub>      | Aflatoxin B <sub>1</sub>          |
| AFB <sub>1</sub> -Lys | Aflatoxin B <sub>1</sub> -lysine  |
| BMI                   | Body mass index                   |
| ELISA                 | Enzyme-linked immunosorbent assay |
| PSM                   | Propensity score matching         |
| SMD                   | Standardized mean difference      |

## Declarations

**Competing Interests:** The authors declare that they have no competing interests.

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Guangzhou Center for Disease Control and Prevention (approval Nos. [GZCDC-ECHR-2026P0030], [GZCDC-ECHR-2026P0031], [GZCDC-ECHR-2026P0032]). All participants were fully informed of the study objectives and procedures, and written informed consent was obtained prior to questionnaire interviews and blood sample collection.

### Data availability

The datasets analysed during the current study are not publicly available due to data ownership and confidentiality restrictions imposed by the Guangzhou Center for Disease Control and Prevention (Guangzhou Health Supervision Institute), but are available from the corresponding author on reasonable request and with permission from the data owner.

### CRedit authorship contribution statement

Lili Huang: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. Yanyan Wang: Conceptualization, Project administration. Yan Li: Conceptualization, Project administration. Jiangbo Lei: Investigation, Validation. Tianji Lin: Investigation, Validation. Jinchang Zhou: Investigation, Writing – review & editing. Yuzhe Wu: Investigation, Writing – review & editing. Zhijun Bai: Conceptualization, Project administration. Zhoubin Zhang: Conceptualization, Project administration. Weiwei Zhang: Conceptualization, Project administration, Supervision, Funding acquisition.

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Figures

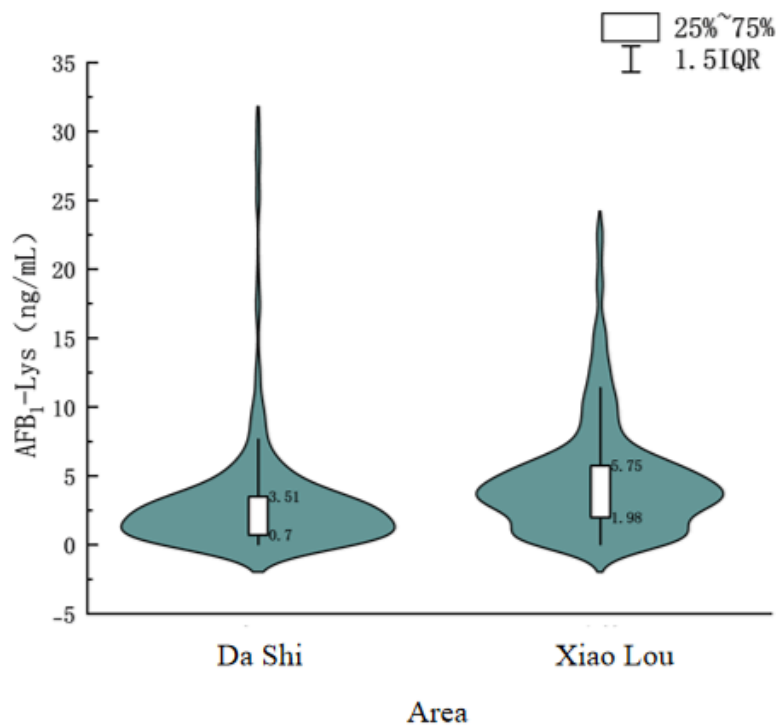


Figure 1

Grouping violin plot of serum AFB<sub>1</sub> adducts (ng/mL) among residents in two regions over three years

Note: 25% to 75%: This is the interquartile range, representing the 25th percentile to the 75th percentile.