The association of isocarbophos and isofenphos with different types of glucose metabolism: the role of inflammatory indicators

Running Title: Isocarbophos, isofenphos, inflammatory indicators, and glucose metabolism

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Abstract

Aims

Our investigation focused on the associations between isocarbophos and isofenphos with impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM), as well as how much of these associations might be accounted for by markers of inflammation.

Methods

There were 2701 participants in a case-control study. Plasma isocarbophos and isofenphos concentrations were measured using gas chromatography and triple quadrupole tandem mass spectrometry. Generalized linear models were used to calculate the relationships between plasma isofenphos and isocarbophos levels with inflammatory factor levels and T2DM. Inflammatory indicators were used as mediators to estimate the mediating effects on the above associations.

Results

Isocarbophos and isofenphos were positively related with T2DM after adjusting for other factors. The odds ratio (OR) (95% confidence interval (CI)) for T2DM was 4.1% (OR (95% CI): 1.041 (1.015, 1.068)) and the odds ratio (95% CI) for IFG was 6.6% (OR (95% CI): 1.066 (1.009, 1.127)) per unit rise in ln-isocarbophos. The incidence of T2DM increased by 6.4% for every 1 unit more of ln-isofenphos (OR (95% CI): 1.064 (1.041, 1.087)). Additionally, a 100% rise in ln-isocarbophos was linked to 3.3% higher ln-HOMA2IR and a 0.029 mmol/L higher glycosylated hemoglobin A1c (HbA1c) (95% CI: 0.007, 0.051). While a 100% rise in ln-isofenphos was linked to increases in ln-HOMA2 (95% CI: 1.6%, 5.2%) and ln-HOMA2IR (95% CI: 3.6%, 8.1%) of 5.8% and 3.4%, respectively. Furthermore, white blood cell (WBC) and neutrophilic (NE) were found to be mediators in the relationship between isocarbophos and T2DM, and the corresponding proportions were 17.12% and 17.67%, respectively.

Conclusion

Isofenphos and isocarbophos are associated with IFG and T2DM in the rural Chinese population, and the inflammatory indicators (WBC and NE) have a significant role in this relationship.

1. Introduction

High blood glucose levels are a defining feature of diabetes mellitus, a chronic metabolic disorder. Over 400 million individuals worldwide currently have type 2 diabetes mellitus (T2DM), and over the next 30 years, this figure is projected to drastically rise[1–3], even to epidemic proportions[4]. Although T2DM mainly occurred in middle-aged and elderly, the supreme relative increase in the incidence and prevalence of T2DM can’t be ignored since 2000 in younger people (those < 40 years of age), as well as adolescents and even children[5]. Data from the World Health Organization (WHO) show that age-standardized T2DM prevalence is
increasing in China, where the overall incidence of T2DM is 9.4%[1]. T2DM can severely damage the heart, blood vessels, eyes, kidneys[6], and nerves over time. Moreover, there can be serious complications[7–9]. The transition from impaired fasting glucose (IFG) to diabetes can take many years; however, most individuals with IFG (perhaps as high as 70%) will eventually progress to diabetes[10]. T2DM and IFG are huge global problems, not only in terms of health but also in epidemiological and economic terms[11]. In addition, it is believed that the interplay of environmental, biological, and behavioral risk factors leads to T2DM, with studies showing that air pollution alters vascular endothelial function and triggers inflammation and insulin resistance[12]. For populations in rural areas, the use of pesticides is an important contributor to air pollution.

The use of pesticides has also been gradually increasing in recent years. In 2018, approximately 300,000 tonnes of pesticides were applied in China[13]. Organophosphorus pesticides (OPs) account for one-third of all other agrochemicals used[14]. In addition, more than 99.9% of pesticides end up in the environment, causing serious environmental pollution and severe risks of human exposure[13, 15]. Numerous research have shown that the high use of OPs prejudicial to health[16, 17]. Xianwei Guo[18] et al. showed a modest association between levels of OPs metabolites and the prevalence of T2DM in US adults. Several case reports and animal tests involving examination have confirmed that OPs stimulation interferes with glucose homeostasis[19]. But the existing concern of OPs were mostly malathion and chlorpyrifos, etc. Isocarbophos (O-2-isopropoxy carbonyl phenyl O-methyl phosphoramidothioate)[20], a broad-spectrum OPs, has been widely used in China since 1980 as an acetylcholinesterase inhibitor to control agricultural pests including locusts[21]. It is produced in quantities of up to 5000 tonnes per year and used on such a large scale, thereby lead to significant environmental risks[20]. In China, isocarbophos is still widely used in cotton and rice cultivation[22], although it was prohibited in vegetables and fruits due to its toxicity[23]. Isofenphos (O-ethyl-O-2-isopropoxy carbonyl phenyl-N-isopropyl phosphoroamidothioate) is also one of the most widely used OPs[24] and can also cause cholinergic toxicity in humans. The use of isocarbophos and isofenphos on such a large scale must have dramatic effects on humans, especially for farm workers and their families with increased exposure or for individuals living near fields where pesticides are used[25]. However, studies on isocarbophos and isofenphos with glucose metabolism are scarce, the direct evidence is unclear, and further exploration is needed.

Chronic low-grade inflammation is recognized to play a role in numerous disease processes associated with insulin resistance and obesity[26], which is a critical prior pathophysiological feature of T2DM[27]. Recent developments in genome-wide association studies (GWAS) suggested that inflammation may be related to the prevalence of T2DM in people, even if the role of chronic inflammation in the development of insulin resistance and T2DM has not yet been proven in humans[28]. In addition, a growing number of studies have shown that OPs can cause inflammation except for inhibiting cholinesterase activity[29–31], but few studies have examined the role of inflammatory factors in the relationship between OPs and T2DM.

In summary, the direct evidence of the relationship between the OPs (isofenphos and isocarbophos) and T2DM remains limited, and the role of inflammatory indicators in this relationship is unclear and potentially complex. Therefore, we conducted a case-control study of 2701 adult rural residents in China to explore the relationships between isofenphos and isocarbophos with T2DM and IFG. In addition, we further explored the possible mediating role of inflammatory factors in these relationships.
2. Material And Methods

2.1 Participants in the study

The Henan Rural Cohort, a sizable prospective cohort, provided the data for this investigation. Its main objective was to investigate the incidence of chronic non-communicable diseases in a rural Chinese community and the underlying risk factors for such diseases. A detailed description of this cohort has been mentioned in previous article[32]. We used 925 T2DM patients in the current study to conduct a case-control study. And 925 controls with normal glucose tolerance (NGT) and 925 with impaired fasting glucose (IFG) were randomly selected by matching T2DM patients in the same population based on age (± 3) and gender. Of the 2775 study subjects selected, 41 individuals were deleted for lack of pesticide data, a total of 33 were excluded due to extreme values of hypersensitive C-reactive protein (hsCRP) (n = 28), lack of white blood cell (WBC) data (n = 4), and lack of neutrophilic (NE) data (n = 1). 2701 study subjects were eventually included in this study: 896 patients with T2DM, 905 subjects with NGT, and 900 patients with IFG. This study complied with the Declaration of Helsinki. Each participant in the study submitted written informed consent, and Zhengzhou University’s Life Sciences Ethics Committee granted ethical approval (ethical approval number: [2015] MEC (S128)).

2.2 Assessment of outcomes

The diagnostic criteria for IFG and T2DM are recommended by the American Diabetes Association (ADA) (2002) and the WHO (1999) guidelines. After excluding type 1 diabetes, gestational diabetes, and other specific types of diabetes, T2DM was defined as having been diagnosed by a physician with T2DM and taking antidiabetic medication or insulin in the past two weeks, having fasting plasma glucose (FPG) ≥ 7.0 mmol/L, or glycosylated hemoglobin A1c (HbA1c) ≥ 6.5%. IFG was defined as 6.1 mmol/L ≤ FPG < 7.0 mmol/L or 5.7% < HbA1c < 6.5%.

2.3 Data collection

A structured questionnaire was used by trained researchers to collect information from face-to-face interviews about sociodemographic traits (such as age, gender, education level, marital status, and income), lifestyle traits (such as drinking, smoking, and physical activity), and family medical history. Not in school, elementary school, middle school, high school, college or university, and above were the categories for educational level. According to an individual’s average monthly income, economic standing was split into five categories: less than 500 renminbi (RMB), 500 to 1000 RMB, 1000 to 2000 RMB, 2000 to 3000 RMB, and more than 3000 RMB (yuan). Marital status was listed as married/cohabiting or widowed, single, divorced/separated. Smoking status was recorded as a smoker (current and former smoker) or non-smoker, with current smoker being defined as smoking at least one cigarette per day for more than six months. Drinking status was recorded as either a drinker (current and former drinker) or a non-drinker, with current drinker being defined as drinking at least 12 times a year. Physical activity levels were classified as low, medium, or high based on the International Physical Activity Questionnaire. Family history of disease was defined as having at least one immediate parent with the disease. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). The homeostasis model assessment (HOMA)-2 of insulin resistance (HOMA2-IR) and β-cell function (HOMA2-
β) were calculated using the updated computer-based HOMA index of insulin resistance (HOMA2-IR) and computer-based HOMA2-β%.

2.4 Laboratory measurements

Participants who had fasted for at least eight hours had venous blood samples drawn, which were then centrifuged at 3000 rpm for 10 minutes to extract serum samples, and stored at -80°C for further analysis. Using an automated biochemical analyzer, FPG was quantified (rocheCobasC501). Enzymatic HbA1c measurements were made with an automated analyzer. Serum hsCRP levels were measured using an immunoturbidimetric method (rocheCobasC501). WBC, NE, lymphocyte (LYM), monocytes (MONO), eosinophil (EO), and basophilic (BASO) levels were obtained from routine blood tests. The concentrations of isocarbophos and isofenphos in plasma were measured using a 7890B gas chromatograph coupled with a 7010B triple quadrupole tandem mass spectrometer (GC-MS/MS) (Agilent, USA) fitted with a DB-5 MS capillary column (Agilent, USA). The injection volume was 1 µL. Helium was used as the carrier gas. The instrument was used in multiple reaction monitoring (MRM) mode for quantitative and qualitative analysis. In the same analytical run, cases and matched controls were measured, although we are unsure of whether the sample was a case or control in the test (blinded).

2.5 Statistical analysis

Categorical variables were expressed as frequencies (%) and continuous variables were expressed as medians (interquartile ranges) (IQR). Wilcoxon rank sum test (continuous variables) and chi-square test (categorical variables) were used to assess the differences in each inflammatory factor and the underlying characteristics between cases and control subjects. OPs (isofenphos and isocarbophos) and each inflammatory factor were subjected to natural logarithmic transformation in subsequent analyses due to their skewed distribution.

To explore potential non-linear and dose-response relationships, isofenphos and Plasma isocarbophos levels were divided into three tertiles according to the levels of control subjects, and the lowest tertile was defined as the reference group. We used generalized linear models to calculate the relationships of isofenphos, isocarbophos levels, and inflammatory factor levels with IFG and T2DM and the odds ratio (ORs) were reported. Trend tests were performed by entering categorical variables as continuous variables in the generalized linear model to assess the relationship between increasing tertile levels. Two models were constructed for this study. Model 1 did not adjust for variables and model 2 was adjusted for age, gender, marital status, education, mean monthly income, smoking status, drinking status, physical activity, family history of T2DM, and BMI.

To determine whether the relationships between isofenphos, isocarbophos levels, and T2DM were mediated by inflammatory factors, we performed a mediation analysis, where the mediators had to be continuous as previously described elsewhere[33], and ran 5000 bootstraps resample by adjusting for the same confounders used in the model 2 described above. The technique required that the independent variable be significantly correlated with the dependent variable (Pathc) and that the proposed mediator be significantly correlated with the independent variable (Patha) and dependent variable (Pathb). The proportion of the effect explained by the mediator (PE) was defined as PE = indirect effect/total effect.
All statistical analyses were performed using SPSS software, version 21.0, and R software version 4.1.1. Two-tailed P values < 0.05 were considered statistically significant.

3. Results

3.1 Characteristics of the study participants

Table 1 summarised the basic characteristics of the study participants by case-control status. The differences in the family history of T2DM, BMI, FPG, HbA1c, HOMA2-IR, HOMA2-β, hsCRP, WBC, NE, LYM, MONO, and BASO were statistically significant in all three types of populations (P < 0.05). Among the three groups, patients with T2DM had a family history of T2DM, higher levels of BMI, FPG, HbA1c, HOMA2-IR, hsCRP, WBC, and NE, as well as lower levels of HOMA2-β compared to NGT group and IFG group.
Table 1
Basic characteristic of study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>NGT (n = 905)</th>
<th>T2DM (n = 896)</th>
<th>IFG (n = 900)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, n (%)</td>
<td>346 (38.2%)</td>
<td>342 (38.2%)</td>
<td>350 (38.9%)</td>
<td>0.941</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.059</td>
</tr>
<tr>
<td>Never</td>
<td>674 (74.5%)</td>
<td>663 (74.0%)</td>
<td>680 (75.6%)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>58 (6.4%)</td>
<td>85 (9.5%)</td>
<td>59 (6.6%)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>173 (19.1%)</td>
<td>148 (16.5%)</td>
<td>161 (17.9%)</td>
<td></td>
</tr>
<tr>
<td>Drinking status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.120</td>
</tr>
<tr>
<td>Never</td>
<td>741 (81.9%)</td>
<td>712 (79.5%)</td>
<td>756 (84.0%)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>48 (5.3%)</td>
<td>63 (7.0%)</td>
<td>48 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>116 (12.8%)</td>
<td>121 (13.5%)</td>
<td>96 (10.7%)</td>
<td></td>
</tr>
<tr>
<td>Married/cohabitation, n (%)</td>
<td>804 (88.8%)</td>
<td>803 (89.6%)</td>
<td>797 (88.6%)</td>
<td>0.936</td>
</tr>
<tr>
<td>Widowed</td>
<td>84 (9.3%)</td>
<td>73 (8.1%)</td>
<td>86 (9.6%)</td>
<td></td>
</tr>
<tr>
<td>Divorce/separation</td>
<td>4 (0.4%)</td>
<td>6 (0.7%)</td>
<td>4 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>13 (1.4%)</td>
<td>14 (1.6%)</td>
<td>13 (1.4%)</td>
<td></td>
</tr>
<tr>
<td>Educational levels, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.248</td>
</tr>
<tr>
<td>Illiteracy</td>
<td>241 (26.6%)</td>
<td>224 (25.0%)</td>
<td>224 (24.9%)</td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>279 (30.8%)</td>
<td>270 (30.1%)</td>
<td>275 (30.6%)</td>
<td></td>
</tr>
<tr>
<td>Junior high school</td>
<td>315 (34.8%)</td>
<td>303 (33.8%)</td>
<td>318 (35.3%)</td>
<td></td>
</tr>
<tr>
<td>High School</td>
<td>64 (7.1%)</td>
<td>88 (9.8%)</td>
<td>80 (8.9%)</td>
<td></td>
</tr>
<tr>
<td>College or above</td>
<td>6 (0.7%)</td>
<td>11 (1.2%)</td>
<td>3 (0.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Values are median (inter-quartile range) for continuous variables and number (percentages) for categorical variable.

P values were calculated using the Mann-Whitney U test and chi-square.

Abbreviation: RMB, renminbi; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycosylated haemoglobin A1c; IFG, impaired fasting glucose; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus; hsCRP, hypersensitive C-reactive protein; WBC, white blood cell; NE, neutrophilic; LYM, lymphocyte; MONO, monocytes; EO, eosinophil; BASO, basophilic.
<table>
<thead>
<tr>
<th>Variables</th>
<th>NGT (n = 905)</th>
<th>T2DM (n = 896)</th>
<th>IFG (n = 900)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per capita monthly income, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>&lt; 500, RMB</td>
<td>358(39.6%)</td>
<td>365(40.7%)</td>
<td>371(41.2%)</td>
<td></td>
</tr>
<tr>
<td>500~, RMB</td>
<td>277(30.6%)</td>
<td>253(28.2%)</td>
<td>294(32.7%)</td>
<td></td>
</tr>
<tr>
<td>1000~, RMB</td>
<td>215(23.8%)</td>
<td>213(23.8%)</td>
<td>187(20.8%)</td>
<td></td>
</tr>
<tr>
<td>2000~, RMB</td>
<td>36(4.0%)</td>
<td>44(4.9%)</td>
<td>29(3.2%)</td>
<td></td>
</tr>
<tr>
<td>3000, RMB</td>
<td>19(2.1%)</td>
<td>21(2.3%)</td>
<td>19(2.1%)</td>
<td></td>
</tr>
<tr>
<td>Physical activity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.113</td>
</tr>
<tr>
<td>Low</td>
<td>211(23.3%)</td>
<td>255(28.5%)</td>
<td>237(26.3%)</td>
<td></td>
</tr>
<tr>
<td>Mediate</td>
<td>460(50.8%)</td>
<td>408(45.5%)</td>
<td>438(48.7%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>234(25.9%)</td>
<td>233(26.0%)</td>
<td>225(25.0%)</td>
<td></td>
</tr>
<tr>
<td>Family history of T2DM, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.00(54.00,66.00)</td>
<td>61.00(54.00,66.00)</td>
<td>61.00(54.00,66.00)</td>
<td>0.997</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.44(21.18,25.77)</td>
<td>25.43(23.39,27.61)</td>
<td>24.22(22.04,26.48)</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG(mmol/L)</td>
<td>4.93(4.61,5.22)</td>
<td>7.93(6.93,10.09)</td>
<td>5.29(4.88,5.81)</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1c(%)</td>
<td>5.30(5.10,5.50)</td>
<td>7.40(6.60,8.85)</td>
<td>5.90(5.70,6.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>1.53(1.20,1.94)</td>
<td>2.02(1.56,2.70)</td>
<td>1.64(1.28,2.09)</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA2-β(%)</td>
<td>130.40(106.00,157.300)</td>
<td>60.50(36.25,86.80)</td>
<td>115.95(92.40,146.05)</td>
<td>0.001</td>
</tr>
<tr>
<td>hsCRP(mg/L)</td>
<td>0.97(0.60,1.68)</td>
<td>1.31(0.76,2.66)</td>
<td>1.15(0.68,2.27)</td>
<td>0.001</td>
</tr>
<tr>
<td>WBC(×10⁹ /L)</td>
<td>5.50(4.69,6.50)</td>
<td>6.20(5.28,7.45)</td>
<td>5.90(5.00,7.08)</td>
<td>0.001</td>
</tr>
<tr>
<td>NE(×10⁹ /L)</td>
<td>3.08(2.49,3.84)</td>
<td>3.61(2.91,4.50)</td>
<td>3.27(2.62,4.16)</td>
<td>0.001</td>
</tr>
<tr>
<td>LYM(×10⁹ /L)</td>
<td>1.83(1.48,2.27)</td>
<td>2.02(1.62,2.53)</td>
<td>2.07(1.65,2.50)</td>
<td>0.001</td>
</tr>
<tr>
<td>MONO(×10⁹ /L)</td>
<td>0.30(0.24,0.37)</td>
<td>0.29(0.24,0.37)</td>
<td>0.32(0.26,0.39)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are median (inter-quartile range) for continuous variables and number (percentages) for categorical variable.

P values were calculated using the Mann-Whitney U test and chi-square.

Abbreviation: RMB, renminbi; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycosylated haemoglobin A1c; IFG, impaired fasting glucose; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus; hsCRP, hypersensitive C-reactive protein; WBC, white blood cell; NE, neutrophilic; LYM, lymphocyte; MONO, monocytes; EO, eosinophil; BASO, basophilic.
### 3.2 Association of isocarbophos and isofenphos with IFG and T2DM

The correlations between isocarbophos and isofenphos with IFG and T2DM were depicted in Fig. 1. Per 1 unit rise in ln-isocarbophos, after controlling for other variables, there was a 4.1% (OR (95% CI): 1.041 (1.015, 1.068)) and a 6.6% (OR (95% CI): 1.066 (1.009, 1.127)) increased risk of T2DM and IFG, respectively. Only the third tertile was linked to a 12.4% (OR (95% CI): 1.124 (1.064, 1.188) and 13.8% (OR (95% CI): 1.138 (1.017, 1.274)) greater risk of T2DM and IFG, respectively, as compared to the first tertile.

Additionally, there was a 6.4% greater risk of T2DM per 1 unit rise in ln-isofenphos (OR (95% CI): 1.064 (1.041, 1.087)). In comparison to tertile 1, the second and third tertiles of ln-isofenphos were linked to a 42% and 13.6% (OR (95% CI): 1.420 (1.344, 1.501) and 1.136 (1.069, 1.207) greater risk of T2DM, respectively. This association exhibited a trend of the risk rising with the level of isofenphos (P for trend  0.05). After controlling for a number of factors, the relationships between isofenphos and IFG were not statistically significant. Supplementary Table 1 had comprehensive data.

### 3.3 Relationship of isocarbophos and isofenphos with FPG, HbA1c, HOMA2-IR, and HOMA2-β

The connections between isocarbophos and isofenphos with markers of glucose metabolism in the NGT group were shown in Fig. 2 and Supplementary Table 2. After various factors were taken into account in model 2, a 100% rise in ln-isocarbophos was linked to 3.3% higher ln-HOMA2IR and a 0.029 mmol/L higher HbA1c (95% CI: 0.007, 0.051). Similar to this, only the third tertile of ln-isocarbophos was linked to a 7.6% higher ln-HOMA2IR and a 0.064 mmol/L higher HbA1c (95% CI: 0.020, 0.109). According to model 2, a 100% rise in ln-isofenphos was linked to increases in ln-HOMA2 and ln-HOMA2IR of 3.4% and 5.8%, respectively. Equally, ln-HOMA2IR was 9.3% (95% CI: 3.3%, 15.2%) and 9.3% (95% CI: 3.2%, 15.3%) higher in the second and third tertiles of ln-isofenphos than in tertile 1, respectively. However, only the third tertile of ln-isofenphos was associated with 7.4% (95% CI: 2.7%, 12.3%) higher ln-HOMA2-β versus tertile 1. The relationships between isocarbophos and isofenphos with glucose metabolism indicators in T2DM and IFG groups were shown in Supplementary Figs. 1 and 2. Similar results were found in the T2DM and IFG groups.
3.4 Association of isocarbophos and isofenphos with indicators of inflammation

The associations between isocarbophos and isofenphos with the indicators of inflammation were demonstrated in Fig. 3 and Supplementary Table 3. Each 1-unit increase in ln-isocarbophos was associated with increases in WBC and NE levels of 1.8% and 2.2% in the T2DM group, respectively, after controlling for all confounding variables. The associations between ln-isocarbophos and inflammatory indicators were not significant after adjusting multiple variables in the IFG group. Surprisingly, the relationship between ln-isofenphos and the indicators of inflammation was not significant in either the T2DM or IFG populations.

3.5 Relationship between inflammatory indicators and IFG and T2DM

As shown in Table 2, the findings revealed that inflammatory indicators were associated with IFG and T2DM. For instance, in the adjusted model, the ORs (95% CIs) of WBC with T2DM and IFG were 1.432 (1.321, 1.552) and 1.753 (1.470, 2.090), respectively. The OR (95% CI) of NE with T2DM and IFG were 1.343 (1.262, 1.428) and 1.319 (1.153, 1.508), respectively.

Table 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>T2DM (OR and 95%CI)</th>
<th>IFG (OR and 95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>hsCRP</td>
<td>1.110 (1.080, 1.141)</td>
<td>1.060 (1.031, 1.089)</td>
</tr>
<tr>
<td>WBC</td>
<td>1.532 (1.412, 1.662)</td>
<td>1.432 (1.321, 1.552)</td>
</tr>
<tr>
<td>NE</td>
<td>1.397 (1.312, 1.487)</td>
<td>1.343 (1.262, 1.428)</td>
</tr>
<tr>
<td>LYM</td>
<td>1.216 (1.137, 1.300)</td>
<td>1.151 (1.078, 1.229)</td>
</tr>
<tr>
<td>MONO</td>
<td>0.941 (0.881, 1.007)</td>
<td>0.914 (0.856, 0.978)</td>
</tr>
<tr>
<td>EO</td>
<td>1.015 (0.987, 1.043)</td>
<td>1.005 (0.978, 1.032)</td>
</tr>
<tr>
<td>BASO</td>
<td>1.032 (1.007, 1.058)</td>
<td>1.025 (1.001, 1.049)</td>
</tr>
</tbody>
</table>

Model 1: unadjusted. Model 2: age, sex, BMI, physical activity, education level, smoking status, drinking status, marry status, family history of diabetes, Per capita monthly income

Abbreviation: IFG, impaired fasting glucose; T2DM, type 2 diabetes mellitus; hsCRP, hypersensitive C-reactive protein; WBC, white blood cell; NE, neutrophilic; LYM, lymphocyte; MONO, monocytes; EO, Eosinophil; BASO, basophilic; CI, confidence interval; OR, odds ratio.

3.6 The role of inflammatory indicators WBC and NE in the association between isocarbophos and T2DM

In the investigation just mentioned, we discovered that there was a substantial relationship between isocarbophos level with WBC, NE, and T2DM. In order to evaluate the potential mediating effects of WBC and
NE on the associations between isocarbophos and T2DM, we also conducted a mediation study. The associations between isocarbophos and T2DM were strongly mediated by WBC and NE, as shown in Fig. 4. The proportions of the mediation effect of WBC and NE for T2DM were 17.12% and 17.67%, respectively.

4. Discussion

This case-control study showed a positive correlation between isofenphos, isocarbophos levels with T2DM as well as its related indicators after adjusting for potential confounders. In other words, a higher risk of T2DM was linked to higher levels of isofenphos and isocarbophos. The results of the mediation analysis indicated that WBC and NE were important mediators of the correlation between isocarbophos levels and T2DM, suggesting that systemic inflammation played a crucial role in the relationship between isocarbophos and T2DM.

The relationship between T2DM and OPs had been demonstrated in many studies. Xianwei Guo[18] et al. used three statistical methods to explore the combined effect of multiple OPs metabolites on T2DM, and found that OPs metabolite levels was modestly associated with T2DM prevalence in US adults. A study by Magdalena Czajka[34] et al. discovered that exposure to OPs was linked to metabolic changes related to obesity and T2DM, which raises the possibility that such exposure may enhance vulnerability or risk to other connected elements. Raafat, N[35] et al. found a strong correlation between blood levels of malathion (a kind of OPs) and insulin resistance among farmers. A study by Malekirad[36] et al. showed that farmers with occupational exposure to OPs were susceptible to T2DM. They measured the toxicity of OPs in 187 farmers working with OPs and found that FPG and oral glucose tolerance test (OGTT) were significantly higher in workers exposed to OPs. These studies supported our findings to some extent. But some of the mechanisms linking OPs exposure and T2DM remain controversial. On the one hand, exposure to OPs affected the key pancreatic enzyme for insulin secretion (glutamate dehydrogenase (GDH)). OPs inhibited GDH activity leading to reduced insulin release and then contributing to the development of T2DM. On the other hand, glycogenolysis and gluconeogenesis are also considered to be two key mechanisms associated with OPs-induced hyperglycemia. Some investigators have reported a decrease in hepatic glycogen content following OPs exposure and that hyperglycemia following OPs exposure is accompanied by increased activity of hepatic phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, tyrosine aminotransferase, and hepatic glycogen phosphorylase (GP), which confirms the above hypothesis[37–41]. Therefore, the mechanism of OPs contributing to T2DM is unclear and needs to be further explored.

It had also been shown that repeated low-level exposures could also trigger an inflammatory response[42]. Currently, the mechanisms by which OPs induced inflammation were unclear. One of the most cited was that OPs induced oxidative stress, which was one of the main causes of systemic inflammation, and that damage caused by oxidative stress can activate inflammatory responses[29, 30, 43–45]. Another hypothesis was that the inflammatory response was stimulated through the cholinergic anti-inflammation pathway (CAP), a parasympathetic pathway designed to reduce the inflammatory response through vagal activity, which is inhibited by exposure to OPs[46, 47]. OPs stored in fat appear to induce the release of pro-inflammatory hormones, which stimulated inflammatory messengers, while pesticides in the digestive tract also disrupted the microbiota, thereby promoting increased intestinal permeability, allowing bacteria to enter the body and cause inflammation[29]. These findings supported our findings to some extent.
Inflammation was a natural response of the immune system to injury or infection, but it has the potential to be harmful if not strictly controlled[30]. Moreover, numerous studies have proven that inflammation was one of the major risk factors for metabolic diseases and higher levels of inflammatory mediators were associated with a higher risk of developing T2DM[48, 49]. Additionally, inflammatory cells may alter insulin signalling pathways, promote insulin production, decrease tissue insulin sensitivity, and result in systemic insulin resistance.[50–55]. The current investigation also demonstrated that higher amounts of inflammatory markers were a mediator of the link between OPs and T2DM. In line with the suggestion in one study that OPs-induced insulin resistance may be mediated by inflammatory pathways[55], Gangemi, S.[56] et al. showed in their study that OPs could attenuate proinsulin action through lipotoxic effects, inflammatory stimulation, and induction of oxidative stress, thereby producing insulin resistance and ultimately leading to the development of T2DM.

This study has several strengths, the first is the large sample size and the wide age range. The second is that we have explored through mediation analysis whether the indicators of inflammation mediate the relationship between OPs exposure and different glucose metabolic states. But there are some restrictions that need to be acknowledged. First of all, because this was a case-control study, a causal association may not be able to be established. Second, we may have missed the presence of more OPs because we only tested two, isofenphos and isocarbophos. The study’s subjects were from rural China, therefore due to potential socioeconomic and genetic disparities with urban and other ethnic populations, these results may not apply to them.

5. Conclusion

Isofenphos and isocarbophos were found to significantly enhance the risk of T2DM in this investigation. WBC and NE might be involved in mediating the observed connection between isofenphos and isocarbophos with T2DM. This highlighted the potential for systemic inflammation to play a role in the connection between isofenphos and isocarbophos and type 2 diabetes. For these findings to be confirmed and the underlying mechanisms to be clarified, additional research is urgently required.

Abbreviations

T2DM, type 2 diabetes mellitus; WHO, World Health Organization; Ops, Organophosphorus pesticides; GWAS, genome-wide association studies; NGT, normal glucose tolerance; IFG, impaired fasting glucose; hsCRP, hypersensitive C-reactive protein; WBC, white blood cell; NE, neutrophilic; ADA, American Diabetes Association; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin A1c; RMB, renminbi; BMI, body mass index; HOMA2-IR, (HOMA)-2 of insulin resistance; HOMA2-β, (HOMA)-2 of β-cell function; LYM, lymphocyte; MONO, monocytes; EO, Eosinophil; BASO, basophilic; IQR, interquartile range; OR, odds ratio; WQS, weighted quantile sum regression; BKMR, Bayesian kernel machine regression; GTT, glucose tolerance test; GDH, glutamate dehydrogenase; GP, glycogen phosphorylase; CAP, cholinergic anti-inflammation pathway.

Declarations

Conflict of Interest
The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Sources of Funding

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Availability of data and materials

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Dandan Wei], [Lulu Wang] and [Jintian Geng]. The first draft of the manuscript was written by [Jintian Geng] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Zhengzhou University's Life Sciences Ethics Committee granted ethical approval (ethical approval number: [2015] MEC (S128)).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

References


Figures
Figure 1

The relationship between isocarbophos and isofenphos with IFG and T2DM
Figure 2

The relationship between isocarbophos and isofenphos with glucose metabolism indicators in NGT group
Figure 3

The relationship between isocarbophos and isofenphos with inflammatory indicators

Figure 4

Indirect effect: OR(95%CI) 1.032(1.007,1.059)
Proportion mediated: 17.12%

Indirect effect: OR(95%CI) 1.033(1.007,1.061)
Proportion mediated: 17.67%
Mediation analysis of the relationship between isocarbophos with T2DM by inflammatory indicators

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