ORIGINAL RESEARCH

Risks of Developing Diabetes and Hyperglycemic Crisis Following Carbon Monoxide Poisoning: A Study Incorporating Epidemiologic Analysis and Animal Experiment

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Purpose: Carbon monoxide (CO) poisoning may damage the pancreas, but the effects of CO poisoning on the development of diabetes and on existing diabetes remain unclear. We conducted a study incorporating data from epidemiologic analyses and animal experiments to clarify these issues.

Methods: Using the National Health Insurance Database of Taiwan, we identified CO poisoning patients diagnosed between 2002 and 2016 (CO poisoning cohort) together with references without CO poisoning who were matched by age, sex, and index date at a 1:3 ratio. We followed participants until 2017 and compared the risks of diabetes and hyperglycemic crisis between two cohorts using Cox proportional hazards regressions. In addition, a rat model was used to assess glucose and insulin levels in blood as well as pathological changes in the pancreas and hypothalamus following CO poisoning.

Results: Among participants without diabetes history, 29,141 in the CO poisoning cohort had a higher risk for developing diabetes than the 87,423 in the comparison cohort after adjusting for potential confounders (adjusted hazard ratio [AHR]=1.23; 95% confidence interval [CI]: 1.18–1.28). Among participants with diabetes history, 2302 in the CO poisoning cohort had a higher risk for developing hyperglycemic crisis than the 6906 in participants without CO poisoning (AHR = 2.12; 95% CI: 1.52–2.96). In the rat model, CO poisoning led to increased glucose and decreased insulin in blood and damages to pancreas and hypothalamus.

Conclusion: Our epidemiological study revealed that CO poisoning increased the risks of diabetes and hyperglycemic crisis, which might be attributable to damages in the pancreas and hypothalamus as shown in the animal experiments.

Keywords: animal, carbon monoxide poisoning, diabetes, epidemiology, hypothalamus, pancreas

Introduction

Carbon monoxide (CO) is a colorless, tasteless, and odorless gas produced by incomplete combustion of carbon compounds, including fire, engine exhaust, faulty furnaces, and charcoal-burning.[1] CO poisoning is an important public health issue worldwide. In the United States, CO poisoning contributes to about 15,000 intentional poisoning and 50,000 emergency department visits annually, leading to 1319 deaths in 2014 alone.[1] In Taiwan, a total of 25,912 CO poisoning

cases between 1999 and 2012 were diagnosed, with a 1-month mortality rate of 1.6% and a 3-month mortality rate of 5.0%.[2] In Asia, CO poisoning by charcoal-burning is a common method for suicide.[3] In a study conducted in a medical center in Southern Taiwan, suicidal attempts accounted for 91.4% of CO poisoning cases.[3] Another study in Northern Taiwan showed that 49.4% of CO poisoning cases were suicide attempts.[4] In 2005, Taiwan government set up Taiwan Suicide Prevention Center to prevent suicide, including charcoal burning, which contributes to decreased percentage of suicide attempt in CO poisoning since 2007.[2]

The most well-known toxicity of CO is hypoxic injury, which is due to its high affinity to hemoglobin (Hb), about 250-fold of that of oxygen.[1,5] Other mechanisms may involve inflammation, including mitochondrial dysfunction, increased oxidative stress and reactive oxygen species (ROS), etc.[1,5] Neurological and myocardial injuries are the most common sequelae following CO poisoning due to the high oxygen demands of the brain and heart.[1,5] Nationwide studies in Taiwan reported 6.2% incidence of neurological sequelae during a 12-month follow-up and 0.02% incidence of myocardial injury within 1 month of follow-up.[2,6,7] However, CO toxicity may be systemic, not limited to the brain and heart. A nationwide study revealed that CO poisoning was associated with an increased risk of hypothyroidism and argued that injury to the hypothalamic-pituitary-thyroid axis was the cause.[8] Recently, Weaver et al used a clinical dataset to investigate long-term health consequences of CO poisoning.[9,10] They found that the incidence rates of hypothyroidism, bowel disease, and autoimmune disease, as well as later mortality and hospitalization were higher in patients with CO poisoning compared to the controls.[9,10] Glucose homeostasis was regulated by the brain-endocrine pancreas axis, involving glucose-excited and glucose-inhibited neurons mainly located in the brainstem and hypothalamus, as well as β-cells in the pancreas.[11] CO poisoning may lead to injuries to the brain and pancreas, and thus may increase the risk of diabetes. A nationwide study in Taiwan observed an increased risk of diabetes following CO poisoning.[12] However, no epidemiologic or laboratory studies were conducted to verify the finding, despite that injury to the brain-endocrine pancreas axis is a plausible mechanism. Furthermore, the impact of CO poisoning on existing diabetes has not been evaluated. Therefore, we conducted a study incorporating larger epidemiologic data with an animal model to clarify these issues.

Methods
Epidemiologic Study

The Taiwan National Health Insurance Research Database (NHIRD) was used for the epidemiologic study.[13] The NHIRD is generated from Taiwan National Health Insurance, which enrolled nearly all Taiwan's citizens and provided a population-level evidence for clinical decisions and health policy making.[13] It has also become an important basis for developing artificial intelligence in medical care in the future due to the "big data" feature.[13]

Study and Comparison Cohorts

The study cohort (CO poisoning cohort) consisted of patients with CO poisoning who were identified from the NHIRD using International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes of 986, E868, E952, or E982 or International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) code of T58 at the time of either hospitalization or emergency department care between 2002 and 2016.[2,6–8,12,14] The index date was defined as the date when the patient was diagnosed with CO poisoning. In the study of the risk for developing diabetes, members of the CO poisoning cohort who had diabetes history were excluded, and a comparison cohort was then constructed by sampling candidates without diabetes history from the NHIRD who were matched with the non-diabetic CO poisoning cohort members by age, sex, and index date at a 3:1 ratio.[6] Diabetes history was defined as presence of ICD-9-CM code of 250 or ICD-10 of E08-E13 in at least one hospitalization or three ambulatory care visits before the index date. Separate analyses were conducted on patients with diabetes history to evaluate the effects of CO poisoning on the diabetic population. Hyperglycemic crisis (ie, diabetic ketoacidosis or hyperosmolar hyperglycemic syndrome) was used as the indicator of the effects, and thus members of the CO poisoning cohort who had history of hyperglycemic crisis (ICD-9-CM of 250.1 or 250.2 or ICD-10 of E11.0 or E11.1) in any hospitalization were excluded. Likewise, a diabetic comparison cohort of members with diabetes history who did not have history of hyperglycemic

crisis and were matched with the diabetic CO poisoning cohort members by age, sex, and index date at 1:3 ratio was constructed.

The diagnosis of CO poisoning is generally based on the following criteria: (1) confirmed exposure to CO, including elevated carboxyhemoglobin (COHb) levels or ambient CO concentrations, or a source of CO exposure (COHb level may not be elevated if there is a delay in obtaining the blood sample) and (2) presence of CO poisoning-related symptoms, including headache, dizziness, nausea, vomiting, malaise, fatigue, loss of consciousness, confusion, forgetfulness, visual disturbances, cardiac ischemia, and metabolic acidosis (a blood lactate level >2.5 mmol/L or a calculated base excess level <−2.0 mmol/L).[15]

Definitions of Variables

Age was categorized as 18−34, 35−49, 50−64, and ≥65 years old.[6] Underlying comorbidities were identified as the following: (1) hypertension: ICD-9-CM code of 401–405 or ICD-10 code of I10–I16; (2) obesity: ICD-9-CM code of 278 or ICD-10 code of E66; (3) smoking: ICD-9-CM code of 305.1 or ICD-10 code of Z72.0; (4) pre-diabetes: ICD-9-CM code of 790.2 or ICD-10 code of R73; (5) gestational diabetes: ICD-9-CM code of 648.0 or ICD-10 code of O24; (6) polycystic ovary syndrome: ICD-9-CM code of 256.4 or ICD-10 code of E28.2; (7) hyperlipidemia: ICD-9-CM code of 272 or ICD-10 code of E78.5; (8) hyperuricemia: ICD-9-CM code of 790.6 or 274 or ICD-10 code of E79.0; (9) chronic obstructive pulmonary disease: ICD-9-CM code of 490–496 or ICD-10 code of J44; (10) coronary artery disease: ICD-9-CM code of 410–414 or ICD-10 code of I20–I25; and (11) congestive heart failure: ICD-9-CM code of 428 or ICD-10 code of I50. They are the risk factors for diabetes and thus were potential confounders in the present study.[16] Underlying comorbidities were defined as presence of these diagnostic codes in at least one hospitalization or three ambulatory care claims before the index date. This approach may minimize misclassification introduced by defining the outcome based on diagnostic codes on the claims.[13]

Outcome Measurements

The outcomes of interest in the present study are diabetes and hyperglycemic crises. Cases of diabetes were defined as presence of ICD-9-CM code 250 or ICD-10 codes E08–E13 in at least one hospitalization or three ambulatory care visits after the index date. Cases of hyperglycemic crises were defined as presence of diabetic ketoacidosis or hyperosmolar hyperglycemic syndrome (ICD-9-CM code 250.1 or 250.2 or ICD-10 code E11.0 or E11.1) in at least one hospitalization after the index date.

Animal Study

Adult male Wistar rats (8–10 weeks old, weighing 380–420 g) purchased from the BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan) were used for the study. We used only male rats because female rats have menstrual cycles, which may influence hormone modulation and some physiological parameters.[17] The rats were housed in groups of four per cage in a temperature- and humidity-controlled animal facility with a 12-h light-dark cycle (08:00–20:00). Food and water were available ad libitum. We used 10–12 rats in each study group. All experiments involving experimental animals were conducted in accordance with the National Institutes of Health (NIH) guidelines for care and use of laboratory animals.

Rat Model for CO Poisoning

According to the protocol established in previous studies, we exposed the rats to 1000 ppm of CO for 20 min followed by 3000 ppm of CO for another 80 min in a 98-liter (38.1 cm × 50.8 cm × 50.8 cm) Plexiglas chamber.[18] In the CO chamber, a constant flow of CO was set, and the concentration of CO was continuously monitored by a CO analyzer (TPI-708 carbon monoxide analyzer, Songdo-Dong, Yeonsu-Gu Incheon, Korea). We defined COHb ≥40% in the artery blood as the effective level for CO poisoning because it was the threshold of loss of consciousness (ie, acute severe CO poisoning) in human.[19] The protocol (1000 ppm of CO in the air for 20 min followed by 3000 ppm of CO for another 80 min) was found to be the best after repetitive trials to achieve COHb of ≥40%.[20] The rats were removed from the chamber to breathe fresh room air and regain consciousness after the above protocol of exposure.[18] We removed all near-death rats and excluded them from data analyses. About 0.3 mL of artery blood from rat's tail was drawn by Preset™ arterial blood collection syringe coating with heparin (364390, BD, Becton, USA), and the needle was replaced instead of 26 G 1/2

(301321, BD, Becton, USA). Once the blood was collected, sample was immediately preserved in ice bath and analyzed using blood gas analyzer (Nova Biomedical, Waltham, MA, USA) within 15 minutes to detect the level of COHb.

Histopathological Examination of the Pancreatic Tissue by Hematoxylin-Eosin (H&E) Stain

At the end of the experiment (day 28 after CO exposure, CO poisoning-Day 28), the rat was sacrificed, and the pancreas and brain tissues were harvested and subsequently fixed with formalin solution (4%). After fixation, specimens were sequentially dehydrated with graded alcohol of 50% alcohol for 2 h, 70% alcohol for 2 h, 80% alcohol for 2 h, 95% alcohol for 1 h, 100% alcohol for overnight, and finally soaked in xylene for 90 minutes/time for 3 times. At last, specimens were embedded in paraffin, sliced into cuts of 3 μm by disposable microtome blades (08-635-0, ERMA, Kai industries CO., Ltd., Japan) on the micro slide glass (Pro-01, Matsunami, Japan), and stained using H&E staining. We examined pathological changes of the pancreas and hypothalamus using light microscopy and scored the damages according to a system used in previous studies.[21–26]

Immunofluorescence (IF) Staining

Specimens of the pancreas were processed using the same procedure as in H&E staining, including fixation and dehydration, for IF staining. We applied anti-insulin primary antibodies (ab6995, Abcam, USA) at a ratio of 1:100 and goat anti-mouse IgG secondary antibodies at a ratio of 1:400 (A11004, Invitrogen, USA) and then stained the specimens with 4'6-diamidino-2-phenylindole at a ratio of 1:10,000 (DAPI, Invitrogen, USA). After a final wash with phosphate buffered saline, sections were mounted in glycerol and examined using a confocal microscope with individual fluorescent wavelength (DAPI excitation/emission: 350–400/435–485 nm and TRITC excitation/emission:528–553/590–650 nm). We examined six 40× fields per section and recorded the average number of insulin-positive cells in each field.

Analysis of Circulating Level of Glucose, Insulin, and Glucagon

Blood samples were drawn from the tail vein on the 1st, 7th, 14th, 21st, and 28th day after CO exposure. Serum samples were collected using centrifuge at the speed of $1500 \times g$ for 15 min under 4-degree Celsius environment. We measured the level of glucose using a biochemical automatic analyzer (ABBOTT ARCHITECT c16000, Abbott Laboratories, Lake Forest, IL, USA) and the levels of insulin and glucagon using enzyme-linked immunosorbent assay (ELISA) kits (EZRMI-13K, Merck KGaA, Darmstadt, Germany and EZGLU-30K, Merck KGaA, Darmstadt, Germany, respectively) according to the manufacturer's instructions.

Ethical Statements

The epidemiologic study was conducted according to the Declaration of Helsinki and after the approval of the Institutional Review Board (IRB) of the Chi Mei Medical Center. Patients' informed consents were waived because this was a retrospective analysis of an anonymous database. The waiver did not affect the welfare of patients. Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Chi Mei Medical Center (IACUC No.108041101) and performed in accordance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals*. The best effort was made to minimize animal suffering and number of animals sacrificed.

Statistical methods

In the epidemiologic study, we used independent *t*-tests for continuous variables and chi-square tests for categorical variables to evaluate differences in demographic characteristics and underlying comorbidities between two cohorts. The Kaplan–Meier method with a Log rank test was performed to compare the risk of outcomes between two cohorts. We used multivariable Cox proportional hazards regressions to identify independent predictors of the outcomes and evaluate their effects. Stratified analyses according to age, sex, underlying comorbidities, and follow-up period were also conducted. All patients were right-censored to a new-onset outcome, date of death, or end of follow-up date, which is December 31, 2017. We used SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to perform statistical analyses and STATA 14.0 (Stata Corp., College Station, TX, USA) to display Kaplan–Meier curves.

In the animal study, we used Prism (Version 7.01, S GraphPad Software, Inc., CA, USA) to perform statistical analyses. Data were presented as the mean ± standard error of the mean. Differences in pathophysiological scores

between two groups of rats were evaluated using independent *t*-tests. Hormone values and glucose levels were analyzed using one-way analysis of variance for repeated measures. A two-tailed *p* value of <0.05 was considered statistically significant.

Results

Epidemiologic Study

In non-diabetic CO poisoning and comparison cohorts, age subgroup of 18−34 years (44.8%) was the largest, followed by 35−49 years (36.7%), 50−64 years (13.7%), and ≥65 years (4.8%) (Table 1). The proportion of women was 51.7% in both cohorts because of matching. Compared to patients without CO poisoning, patients with CO poisoning had a higher prevalence of hypertension (7.1% vs 6.3%, *p* < 0.001), smoking (0.4% vs 0.2%, *p* < 0.001), chronic obstructive pulmonary disease (3.1% vs 1.9%, *p* < 0.001), coronary artery disease (2.2% vs 1.4%, *p* < 0.001), and congestive heart failure (0.6% vs 0.3%, *p* < 0.001).

Cox proportional hazards regressions showed that CO poisoning was an independent predictor for diabetes after adjustment for hypertension, obesity, smoking, pre-diabetes, polycystic ovary syndrome, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, coronary artery disease, and congestive heart failure (adjusted hazard ratio [AHR] = 1.23; 95% confidence interval [CI]: 1.18−1.28) (Table 2). In addition to CO poisoning, older age, male sex, hypertension, obesity, pre-diabetes, polycystic ovary syndrome, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, and congestive heart failure were also independent predictors for diabetes.

Stratified analyses showed that CO poisoning was associated with an increased risk of diabetes in all subgroups of age and sex, as well as those with underlying comorbidities of hypertension, hyperlipidemia, and hyperuricemia (Table 3). CO poisoning was associated with an increased risk of diabetes, and the AHR was 1.58 (95% CI: 1.42−1.77) in the first year, which decreased constantly afterwards but still reached statistical significance even after >6 years of follow-up

Table 1 Comparison of Age, Sex, and Underlying Comorbidities Between Patients with and without Carbon Monoxide (CO) Poisoning

Variable	With CO Poisoning N = 29,141	Without CO Poisoning N = 87,423	*p* value
Age (year)			>0.999
18−34	13,054 (44.8)	39,153 (44.8)	
35−49	10,681 (36.7)	32,039 (36.7)	
50−64	3997 (13.7)	12,003 (13.7)	
≥65	1409 (4.8)	4228 (4.8)	
Sex			
Female	15,078 (51.7)	45,234 (51.7)	>0.999
Male	14,063 (48.3)	42,189 (48.3)	
Underlying comorbidity			
Hypertension	2070 (7.1)	5500 (6.3)	<0.001
Obesity	31 (0.1)	67 (0.1)	0.129
Smoking	126 (0.4)	130 (0.2)	<0.001
Pre-diabetes	20 (0.1)	51 (0.1)	0.537
Gestational diabetes	3 (0.01)	12 (0.01)	0.775
Polycystic ovary syndrome	25 (0.1)	89 (0.1)	0.449
Hyperlipidemia	739 (2.5)	2086 (2.4)	0.145
Hyperuricemia	490 (1.7)	1364 (1.6)	0.152
Chronic obstructive pulmonary disease	913 (3.1)	1623 (1.9)	<0.001
Coronary artery disease	652 (2.2)	1181 (1.4)	<0.001
Congestive heart failure	172 (0.6)	233 (0.3)	<0.001

Note: Data are expressed as n (%).

Table 2 Independent Predictors for Diabetes Identified by Cox Proportional Hazards Regressions

Variable	Crude HR (95% CI)	AHR (95% CI)[a]
Cohort		
Without CO poisoning	1 (reference)	1 (reference)
With CO poisoning	1.20 (1.15−1.25)	1.23 (1.18−1.28)
Age (year)		
18−34	1 (reference)	1 (reference)
35−49	2.63 (2.50−2.76)	2.53 (2.41−2.66)
50−64	5.18 (4.91−5.47)	4.41 (4.17−4.67)
≥65	6.84 (6.37−7.35)	4.75 (4.39−5.14)
Sex		
Female	1 (reference)	1 (reference)
Male	1.22 (1.18−1.27)	1.13 (1.09−1.17)
Underlying comorbidity		
Hypertension	3.79 (3.60−3.99)	1.71 (1.61−1.82)
Obesity	3.75 (2.57−5.47)	2.32 (1.59−3.39)
Smoking	1.40 (0.96−2.06)	1.22 (0.83−1.79)
Pre-diabetes	4.35 (2.62−7.22)	1.80 (1.08−3.00)
Polycystic ovary syndrome	1.21 (0.67−2.19)	2.17 (1.20−3.91)
Hyperlipidemia	3.50 (3.23−3.79)	1.35 (1.24−1.48)
Hyperuricemia	3.19 (2.91−3.50)	1.61 (1.47−1.78)
Chronic obstructive pulmonary disease	2.07 (1.87−2.28)	1.19 (1.08−1.32)
Coronary artery disease	3.24 (2.93−3.57)	1.03 (0.93−1.15)
Congestive heart failure	4.41 (3.62−5.36)	1.58 (1.29−1.93)

Notes: [a]Adjusted for hypertension, obesity, smoking, pre-diabetes, polycystic ovary syndrome, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, coronary artery disease, and congestive heart failure.
Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio; CI, confidence interval; CO, carbon monoxide.

(AHR = 1.15; 95% CI: 1.08−1.23). The Kaplan-Meier's method and Log rank test also showed an increased risk of diabetes in the CO poisoning cohort ($p < 0.001$) (Figure 1).

The CO poisoning cohort members with diabetes history had an increased risk of hyperglycemic crisis (AHR = 2.12; 95% CI: 1.52−2.96) compared with diabetic participants without CO poisoning (Table 4). In addition, stratified analyses showed an increased risk in patients with CO poisoning in subgroups of all age except age ≥65 years, both sexes, and with underlying hypertension. Moreover, the Kaplan-Meier's method and Log rank test showed an increased risk of hyperglycemic crisis in the CO poisoning cohort members with diabetes compared to patients with diabetes but without CO poisoning (Figure 2).

Animal Study

Compared to the sham group, CO poisoning rats (CO poisoning-Day 28) had more acinar cell dedifferentiation and necrosis, edema, loss of lobular integrity, parts of inflammation, and perivascular immune cell infiltration (Figure 3). CO poisoning rats showed structural disruption and vacuolation in the islet of Langerhans region (Figure 3A) and had higher damage scores in the comprehensive assessment (Figure 3B). The islet of Langerhans consists of five types of cells and secretes the following principal hormones: insulin (β-cells), glucagon (α-cells), somatostatin (δ-cells), pancreatic polypeptide (PP cells), and ghrelin (ε-cells).[27] Insulin and glucagon are released directly into the blood circulation to regulate glucose levels.[27] Because disruption of the pancreatic islet was observed, we examined the levels of glucose, insulin, and glucagon in the blood. CO poisoning rats had increased glucose levels after CO poisoning-Day 7 and decreased insulin levels on CO poisoning-Days 1, 7, 14, 21, and 28 (Figure 3C and D). However, differences in the glucagon level between the CO poisoning group after CO poisoning and the sham group did not reach statistical significance (Figure 3E). IF staining showed that the sham group had sufficient insulin in the

Table 3 Comparison of the Risk for Developing Diabetes Between Patients with and without Carbon Monoxide (CO) Poisoning Using Cox Proportional Hazards Regressions

Variables	Patients with CO Poisoning n = 29,141			Patients without CO Poisoning (Reference) n = 87,423			Crude HR (95% CI)	AHR (95% CI)[a]
	Case (%)	PY	Rate[b]	Case (%)	PY	Rate		
Overall analysis	3019 (10.4)	207,832.6	14.5	8248 (9.4)	681,255.8	12.1	1.20 (1.15–1.25)	1.23 (1.18–1.28)
Stratified analysis								
Age (year)								
18–34	653 (5.0)	103,039.5	6.3	1778 (4.5)	326,552.6	5.4	1.17 (1.07–1.28)	1.17 (1.07–1.28)
35–49	1347 (12.6)	75,750.9	17.8	3491 (10.9)	252,115.4	13.9	1.29 (1.21–1.38)	1.28 (1.21–1.37)
50–64	738 (18.5)	22,703.0	32.5	2145 (17.9)	78,855.2	27.2	1.02 (1.10–1.30)	1.18 (1.09–1.29)
≥65	281 (19.9)	6339.2	44.3	834 (19.7)	23,732.7	35.1	1.25 (1.09–1.43)	1.21 (1.05–1.38)
Sex								
Female	1512 (10.0)	110,587.7	13.7	3833 (8.5)	355,559.5	10.8	1.27 (1.20–1.35)	1.28 (1.20–1.35)
Male	1507 (10.7)	97,244.9	15.5	4415 (10.5)	325,696.4	13.6	1.15 (1.08–1.22)	1.18 (1.12–1.25)
Underlying comorbidity								
Hypertension	492 (23.8)	10,316.2	47.7	1283 (23.3)	32,665.8	39.3	1.21 (1.09–1.35)	1.21 (1.09–1.34)
Obesity	7 (22.6)	179.6	39.0	20 (29.9)	402.6	49.7	0.80 (0.34–1.90)	0.84 (0.31–2.25)
Smoking	14 (11.1)	673.5	20.8	12 (9.2)	828.5	14.5	1.46 (0.68–3.18)	1.81 (0.73–4.47)
Pre-diabetes	5 (25.0)	71.6	69.9	10 (19.6)	214.4	46.6	1.47 (0.50–4.31)	1.30 (0.35–4.77)
Polycystic ovarian syndrome[c]	<3	–	–	<3	–	–	0.87 (0.19–4.01)	1.10 (0.23–5.21)
Hyperlipidemia	186 (25.2)	3941.4	47.2	470 (22.5)	11,921.9	39.4	1.20 (1.01–1.42)	1.23 (1.03–1.46)
Hyperuricemia	128 (26.1)	2910.2	44.0	347 (25.4)	9316.9	37.2	1.19 (0.97–1.46)	1.24 (1.01–1.52)
Chronic obstructive pulmonary disease	138 (15.1)	5008.0	27.6	268 (16.5)	10,948.0	24.5	1.12 (0.91–1.38)	1.17 (0.95–1.44)
Coronary artery disease	133 (20.4)	3414.2	39.0	272 (23.0)	6926.7	39.3	1.00 (0.81–1.23)	1.08 (0.88–1.34)
Congestive heart failure	44 (25.58)	708.7	62.1	56 (24.0)	1143.5	49.0	1.27 (0.85–1.88)	1.31 (0.87–1.96)
Follow-up period								
<1 year	470 (1.6)	27,861.7	16.9	920 (1.1)	86,735.5	10.6	1.59 (1.42–1.77)	1.58 (1.42–1.77)
<2 years	817 (2.8)	54,008.1	15.1	1792 (2.1)	169,707.5	10.6	1.43 (1.32–1.55)	1.44 (1.32–1.56)
<3 years	1126 (3.9)	77,496.4	14.5	2579 (3.0)	245,457.6	10.5	1.38 (1.29–1.48)	1.39 (1.30–1.49)
<4 years	1403 (4.8)	98,962.3	14.2	3404 (3.9)	315,383.8	10.8	1.31 (1.23–1.40)	1.33 (1.25–1.41)
<5 years	1630 (5.6)	118,347.4	13.8	4204 (4.8)	379,162.5	11.1	1.24 (1.17–1.32)	1.26 (1.19–1.33)
<6 years	1897 (6.5)	135,810.5	14.0	4846 (5.5)	437,011.0	11.1	1.26 (1.19–1.33)	1.28 (1.21–1.35)
≥6 years	1122 (3.9)	72,022.1	15.6	3402 (3.9)	244,244.8	13.9	1.12 (1.05–1.20)	1.15 (1.08–1.23)

Notes: [a]Adjusted for hypertension, obesity, smoking, pre-diabetes, polycystic ovary syndrome, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, coronary artery disease, and congestive heart failure. [b]Per 1000 PY. [c]The database management does not provide count data when the number is smaller than 3 because of confidentiality concerns.
Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio; CI, confidence interval; PY, person-year.
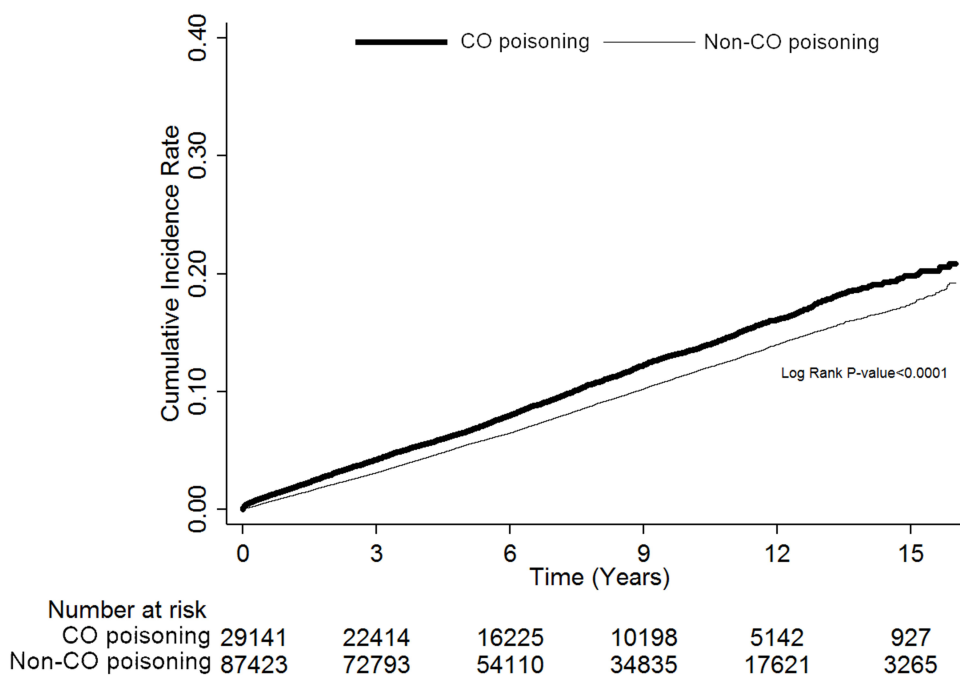
Figure 1 Comparison of the risk for diabetes between patients with and without CO poisoning using Kaplan-Meier's method and Log rank test.
Abbreviation: CO, carbon monoxide.

pancreatic islets (Figure 4A), but CO poisoning rats had insufficient insulin production (Figure 4B). The average number of insulin-positive cells per field on CO poisoning-Day 28 in the CO poisoning group was less than that in the sham group (Figure 4C).

The hypothalamus-pituitary gland axis is well known for its modulatory function of hormonal secretion. Corticotropin-releasing hormone, secreted by the hypothalamus, increases the release of insulin, indicating that functional receptor systems for hypothalamic-releasing hormone agonists exist within the endocrine pancreas.[28] Therefore, we evaluated whether the hypothalamus, an upstream organ, can be injured after CO poisoning. Severe damages such as pyknosis, karyorrhexis, vacuolation, and immune cell infiltration in the hypothalamus of CO poisoning rats were observed in the pathophysiological assessment (Figure 5A). The damage score of the hypothalamus on CO poisoning-Day 28 in the CO poisoning group was higher than that in the sham group (Figure 5B).

Discussion

The epidemiologic study revealed that CO poisoning increased the risk of developing diabetes and increased the risk of hyperglycemic crisis in patients with diabetes. In the animal study, an increased level of glucose and a decreased level of insulin in the blood as well as significant damages in the pancreatic islets and hypothalamus were found, which could explain findings in the epidemiologic study. In addition to CO poisoning, we found that older age, male sex, hypertension, obesity, pre-diabetes, polycystic ovary syndrome, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, and congestive heart failure were independent predictors for diabetes, which is compatible with previous studies.[16]

The most likely cause of the increased risks is CO poisoning-related hypoxic injury, mainly in the beta cells of the pancreatic islets.[29–31] Pancreatic beta cells have high oxygen demand during the process of insulin secretion, making pancreatic beta cells very sensitive to hypoxia.[30] Previous studies showed that hypoxia during islet transplantation contributed to beta cell dysfunction and apoptosis via the mitochondrial cell death pathway.[32,33] Hypoxia induces endoplasmic reticulum stress and upregulates the pro-apoptotic transcription factor C/EBP homologous protein, which initiates apoptotic cell death.[30] Beta cell dysfunction and apoptosis may lead to decreased insulin secretion and subsequent hyperglycemia,[31] which is compatible with the increased risk of developing diabetes in the non-diabetic

Table 4 Comparison of the Risk for Hyperglycemic Crises Between Diabetic Patients with and without Carbon Monoxide (CO) Poisoning Using Cox Proportional Hazards Regressions

Variables	Patients with CO Poisoning n = 2302			Patients without CO Poisoning (Reference) n = 6906			Crude HR (95% CI)	AHR (95% CI)[a]
	Case (%)	PY	Rate[b]	Case (%)	PY	Rate		
Overall analysis	55 (2.4)	12,151.4	4.5	94 (1.4)	43,455.5	2.2	2.07 (1.49–2.89)	2.12 (1.52–2.96)
Stratified analysis								
Age (year)								
18–34	0 (0.0)	117.1	0.0	0 (0.0)	315.9	0.0	–	–
35–49	16 (3.6)	2682.8	6.0	13 (1.0)	9415.8	1.4	4.29 (2.06–8.92)	4.33 (2.08–9.03)
50–64	22 (2.0)	6227.0	3.5	39 (1.2)	22,106.1	1.8	1.97 (1.17–3.32)	2.01 (1.19–3.39)
≥65	17 (2.3)	3124.5	5.4	42 (1.9)	11,617.7	3.6	1.50 (0.85–2.64)	1.52 (0.86–2.67)
Sex								
Female	17 (2.3)	3124.5	5.4	42 (1.9)	11,617.7	3.6	1.85 (1.11–3.07)	1.84 (1.11–3.07)
Male	32 (2.6)	6055.0	5.3	52 (1.4)	22,954.0	2.3	2.28 (1.47–3.54)	2.33 (1.50–3.63)
Underlying comorbidity								
Hypertension	25 (2.1)	5797.4	4.3	45 (1.3)	19,660.7	2.3	1.87 (1.15–3.05)	1.98 (1.21–3.24)
Hyperlipidemia	13 (1.8)	3727.4	3.5	26 (1.1)	13,589.6	1.9	1.84 (0.95–3.58)	1.92 (0.98–3.78)
Hyperuricemia	3 (1.9)	828.6	3.6	7 (1.7)	2460.9	2.8	1.24 (0.32–4.81)	1.29 (0.31–5.39)
Chronic obstructive pulmonary disease	3 (1.4)	1021.8	2.9	6 (1.3)	2531.9	2.4	11.20 (0.30–4.81)	1.03 (0.24–4.47)
Coronary artery disease	7 (1.9)	1715.9	4.1	12 (1.4)	4835.4	2.5	1.64 (0.65–4.18)	1.78 (0.69–4.57)
Follow-up period								
<1 year	16 (0.7)	2113.3	7.6	18 (0.3)	6758.5	2.7	2.82 (1.44–5.54)	2.85 (1.45–5.60)
<2 years	27 (1.2)	3969.5	6.8	29 (0.4)	12,996.7	2.2	3.03 (1.79–5.11)	3.10 (1.84–5.25)
<3 years	33 (1.4)	5528.8	6.0	39 (0.6)	18,423.9	2.1	2.79 (1.76–4.44)	2.88 (1.81–4.58)
<4 years	38 (1.7)	6882.0	5.5	48 (0.7)	23,240.1	2.1	2.64 (1.73–4.05)	2.70 (1.76–4.14)
<5 years	42 (1.8)	8037.9	5.2	61 (0.9)	27,443.9	2.2	2.33 (1.57–3.45)	2.38 (1.61–3.53)
<6 years	43 (1.9)	9021.2	4.8	71 (1.0)	31,087.8	2.3	2.07 (1.42–3.02)	2.12 (1.45–3.09)
≥6 years	12 (0.5)	3130.2	3.8	23 (0.3)	12,367.6	1.9	2.09 (1.04–4.19)	2.15 (1.07–4.33)

Notes: [a]Adjusted for age, sex, hypertension, hyperlipidemia, hyperuricemia, chronic obstructive pulmonary disease, and coronary artery disease. [b]Per 1000 PY. The median duration of diabetes was 5.61 years in patients with CO poisoning and 5.73 years in patients without CO poisoning cohort patients ($p = 0.086$).
Abbreviations: HR, hazard ratio; AHR, adjusted hazard ratio; CI, confidence interval; PY, person-year.
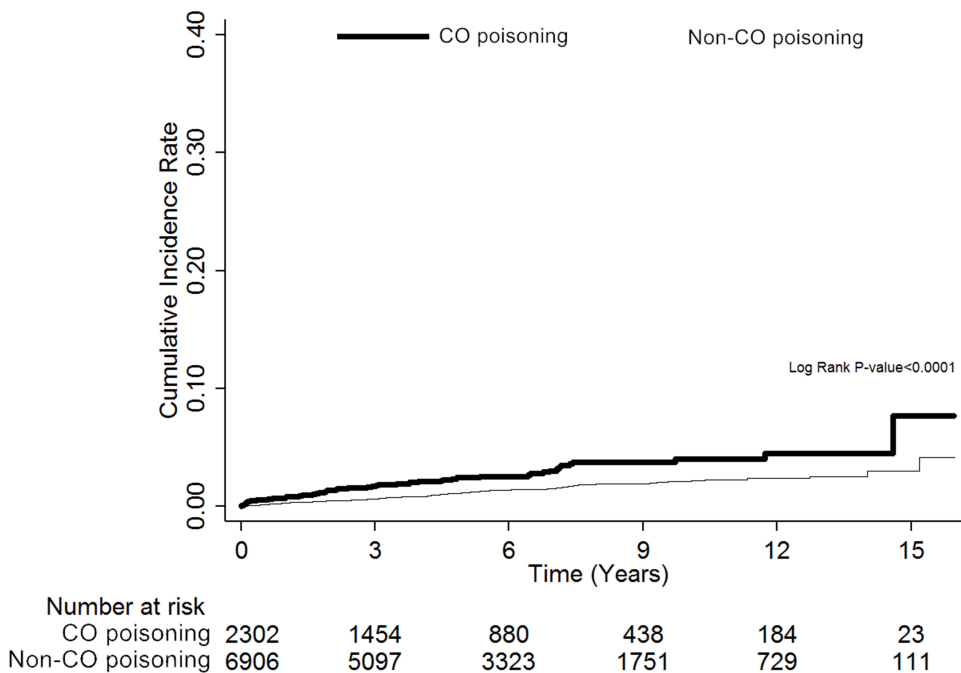
Figure 2 Comparison of the risk for hyperglycemic crises between diabetic patients with and without CO poisoning using Kaplan-Meier's method and Log rank test.
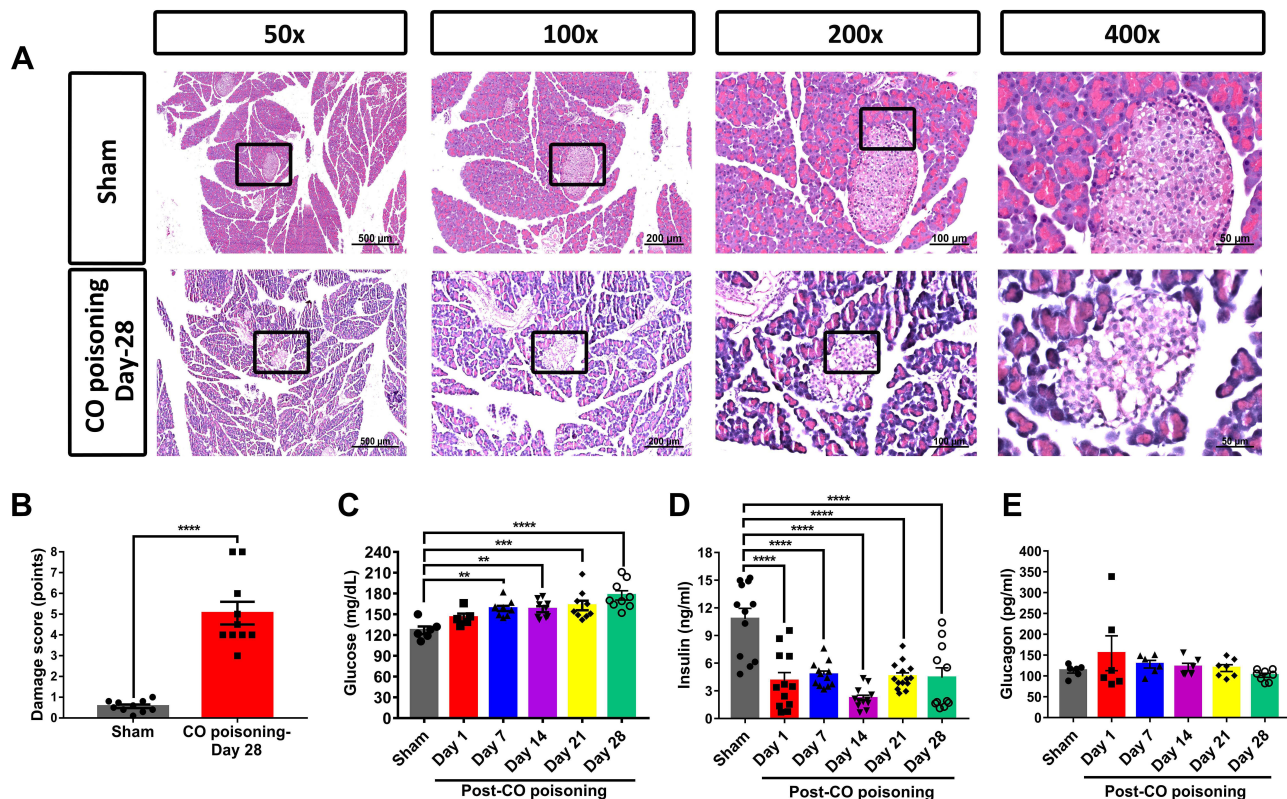**Abbreviation**: CO, carbon monoxide.

Figure 3 Pathological change in the pancreas with different magnification using hematoxylin-eosin stain and relevance of biochemical parameters manifestation. Upper row represents the sham group and the bottom row represents the CO poisoning group on CO poisoning-Day 28 (**A**) and statistical quantification of damage scores between two groups (**B**), n = 10 per group, ****$p < 0.0001$. Biochemical indicator like glucose (**C**) and relative hormone like insulin (**D**) and glucagon (**E**) were detected from the serum by ELISA assay at the indicated day, n = 10–12 per group, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.
Abbreviation: CO, carbon monoxide.
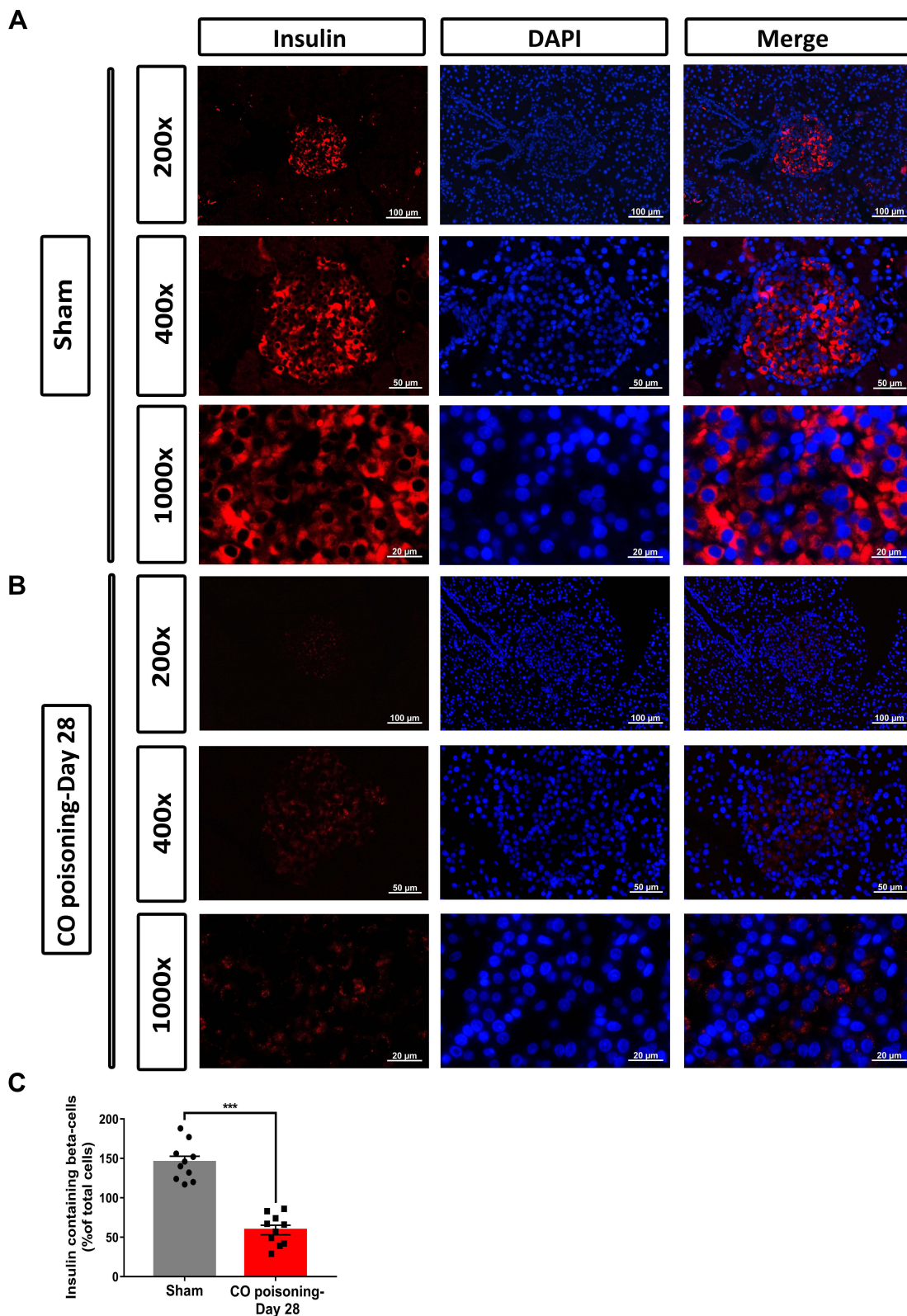
Figure 4 Insulin expression by immunofluorescence staining. Pancreatic islet was labeled by insulin antibody co-staining with nucleus staining (DAPI) shown in different magnification (200×–400×) in the sham (**A**) and CO poisoning group (**B**). Comparison of the number of insulin-positive cells per field was showed in the (**C**) ***$p < 0.001$. **Abbreviation**: CO, carbon monoxide.
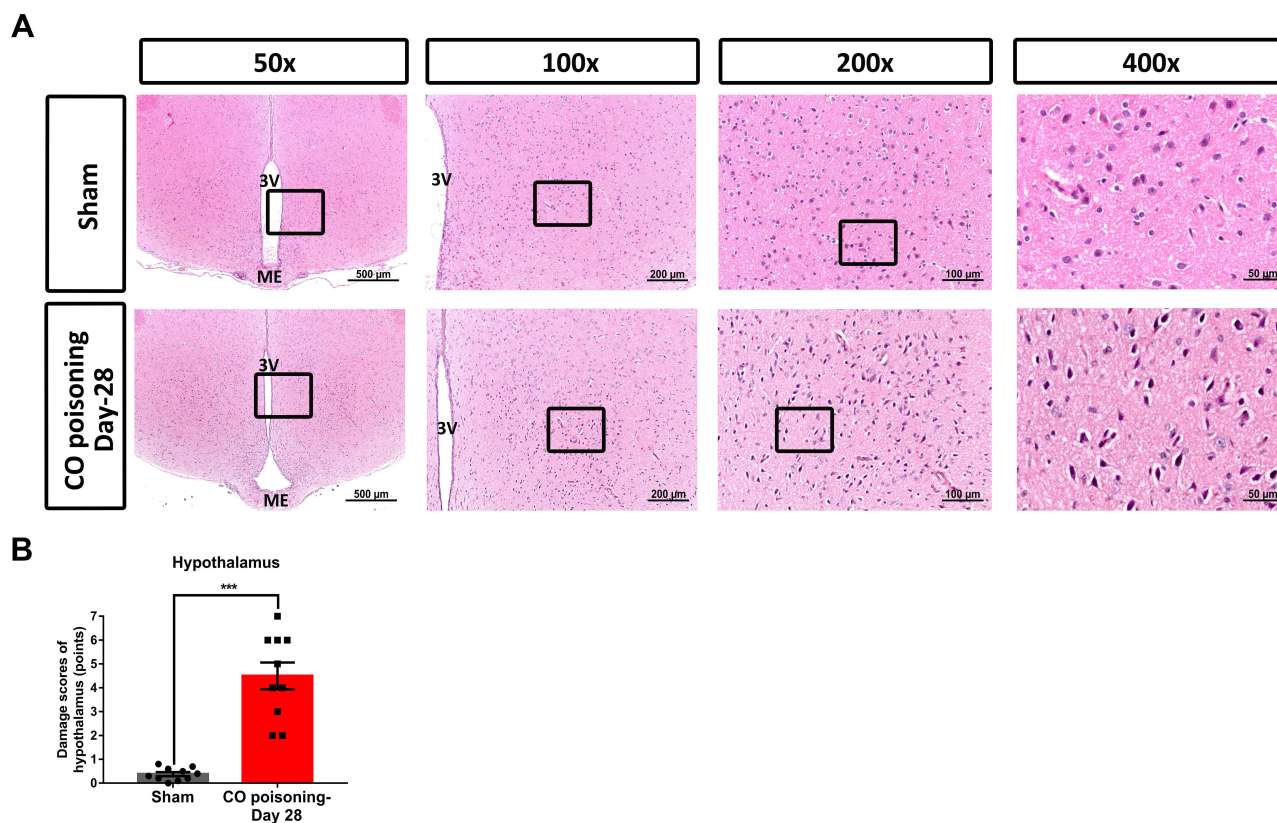
Figure 5 Histopathological change of the brain region by hematoxylin-eosin stain. Significant damages such as pyknosis, karyorrhexis, vacuolation, and immune cell infiltration were found in the hypothalamus of CO poisoning rats (**A**). The damage score of the hypothalamus was significantly higher in the CO poisoning-Day 28 group than that of the sham group (**B**). ***$p < 0.001$.
Abbreviation: CO, carbon monoxide.

population and the increased risk of hyperglycemic crisis in the diabetic population following CO poisoning observed in the present study.

The positive association between CO poisoning and diabetes observed in the present study may help explain the association between smoking and diabetes in previous studies.[34,35] Smoking is a predictor for progression of glucose intolerance, including the progression from normoglycemia to impaired glucose tolerance status and the development of diabetes.[34,35] One of the plausible mechanisms is that CO exposure during smoking leads to hypoxic injuries of the beta cells.[29-31,35] Duration of smoking also has a positive association with the risk of diabetes, and this dose-response relationship suggests a causal link.[35] Although the present study has provided evidence supporting that CO exposure plays a role in the association between smoking and diabetes, the underlying mechanism needs further studies to clarify, because many compounds are involved in smoking such as tar, arsenic, lead, and nicotine.[35]

Mitochondrial dysfunction, ROS generation, and inflammation may also be mechanisms through which CO poisoning increases the risk of diabetes. Patients with diabetes have elevated levels of inflammation markers and mediators, as well as acute-phase reactants (including tumor necrosis factor-alpha, fibrinogen, C-reactive protein, interleukin 6, plasminogen activator inhibitor-1, and sialic acid) and white blood cells.[36] A human study by Chambers et al showed that CO severity did not predict 1 year outcome, supporting the idea that inflammation may be the etiology.[37] A much less severe animal poisoning model would be very informative for this issue. Two major mechanisms related to mitochondrial dysfunction can affect the development and deterioration of diabetes: increased insulin resistance and decreased insulin secretion.[38] Previous human and animal studies revealed impaired oxidative phosphorylation in muscle mitochondria in insulin-resistant states.[39,40] A study using mitochondria isolated from human muscle biopsy specimens observed decreased nicotinamide adenine dinucleotide + hydrogen:O(2) oxidoreductase activity, citrate synthase activity, and smaller skeletal muscle mitochondria in patients with diabetes than in lean volunteers, and concluded that mitochondrial dysfunction in

skeletal muscle is associated with diabetes.[40] Mitochondrial dysfunction affects insulin secretion via impairing adenosine triphosphate production,[38] which is essential for calcium entry and insulin release from storage granules, making it possible for mitochondrial dysfunction and excess ROS to cause oxidative damage and insulin secretion impairment.

The severe damages of the hypothalamus observed in the present study may also elucidate the mechanisms of increased diabetes following CO poisoning. Glucose homeostasis is controlled by glucagon, insulin, and autonomic nervous systems.[11] Activation or inhabitation of glucose-sensing neurons in the hypothalamus may alter glucose homeostasis and contribute to the increased risk of diabetes and poor control of existing diabetes. Direct damage to the pancreas is another mechanism through which CO poisoning may lead to diabetes. A meta-analysis showed that 15% (95% CI: 9%−22%) of the patients with first episode of acute pancreatitis developed new-onset diabetes with 1 year and the relative risk still reached statistical significance after five years of follow up.[44] In the present study, the AHR of diabetes associated CO poisoning was 1.58 (95% CI: 1.42−1.77) in the first year, which still reached statistical significance even after 6 years of follow-up. The findings are compatible with those in the meta-analysis.

Immunological responses following CO poisoning are another possible mechanism of the long-term health effects, including diabetes and other disorders.[41,42] An animal study reported that CO poisoning contributes to adduct formation between myelin basic protein and malonylaldehyde, which causes an immunological cascade and plays a role in delayed neurological sequelae.[41] Compared with the controls, a human study showed that an array of autoantibodies elevated in the patients with CO poisoning.[42] A recent study by Weaver et al showed that autoantibodies and neural proteins were often elevated in the participants with CO poisoning compared with the controls.[43] These studies shed some light on the mechanism and potential biomarker for predicting adverse outcome of CO poisoning.[41–43]

The major strength of the present study is the combination of epidemiologic and animal evidence. In addition, the large sample size of the epidemiologic study allowed adjustment of most potential confounders. The major limitation is the lack of information on some risk factors for diabetes which were not available in the NHIRD, including family history of diabetes and lifestyle such as diet and physical inactivity. However, the animal study, which was not affected by these factors that were not measured in the epidemiologic study, showed increased blood glucose level associated with damages of pancreatic islets and decreased insulin secretion after CO exposure. These findings supported the observations in the epidemiologic study. Second, our animal study may not be representative of CO poisoning in humans. Third, the patients were not coded for subgroups of diabetes, such as type 1, type 2, gestational, etc., and so we were not able to perform further analyses. Further studies to identify which subgroup is the most affected or which is the least affected by CO exposure are warranted.

Conclusion

The epidemiologic data showed that CO poisoning increased the risk of developing diabetes and the risk of hyperglycemic crisis (a sign of poor control of diabetes) in patients with existing diabetes. The most plausible cause is that CO poisoning contributes to hypoxic injuries to the beta cells of the pancreatic islets and subsequently decreases insulin secretion. Other causes, including mitochondrial dysfunction, ROS generation, inflammation, autoantibodies formation, and damage of the hypothalamus may also play a role. Therefore, close follow-up for the development of diabetes, or poor control of existing diabetes are recommended for patients with CO poisoning. The present study may also become an important reference for the research of low-level and long-term exposure of CO, such as those from air pollution, working environment, and tobacco smoke.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

No conflicts of interest to declare.

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