Diabetes mellitus is associated with elevated urinary pyrrole markers of γ-diketones known to cause axonal neuropathy

Xiao Chen,1 Wei Liu,1 Lu Wang,1,2 Dafeng Lin,3 Lulin Nie,1 Kaiwu He,1 Zhiwei Guo,4 Feiqi Zhu,5 Wenting Feng,3 Weimin Liu,6 Jing Yuan,2,7 Xifei Yang,1 Peter Spencer,8,9,10 Jianjun Liu1

ABSTRACT

Introduction Progressive distal symmetrical axonal neuropathy, a complication of diabetes mellitus (DM), has an unknown cause. Normal physiological metabolism and diabetic dysmetabolism are associated with the generation of γ-diketones. γ-Diketones form pyrroles with protein amines, notably with axonal proteins required for the maintenance of nerve fiber integrity, especially elongate, large-diameter peripheral nerve fibers innervating the extremities. We tested the hypothesis that neuropathy-associated γ-diketone pyrroles are elevated in DM.

Research design and methods We measured the urinary concentration of γ-diketone pyrroles in age-matched and gender-matched elderly (60–84 years) persons with (n=267) or without (n=267) indicators of DM based in a community population (9411 community older adults aged ≥60 years) in Shenzhen city, Guangdong, China. We used statistical methods, including a generalized linear model, multivariate logistic regression analysis and restricted cubic splines, to assess linear and nonlinear relationships between urinary γ-diketone pyrroles and indicators of DM.

Results Compared with healthy controls, those with DM had significantly higher levels of fasting blood glucose, glycated hemoglobin A1c, urinary ketone bodies and urinary γ-diketone pyrroles. The median concentration of urinary γ-diketone pyrrole adducts was significantly higher (p<0.0001) in individuals with DM (7.5 (5.4) μM) compared with healthy controls (5.9 (4.3) μM). Both linear and nonlinear relationships were found between urinary γ-diketone pyrroles and indicators of DM.

Conclusions Diabetic dysmetabolism includes increased generation and excretion of neuropathy-associated γ-diketone pyrroles. These findings form the foundation for studies to test whether γ-diketone pyrrole concentration correlates with quantitative sensory (vibration and temperature) and electrodiagnostic testing.

INTRODUCTION

Diabetes mellitus (DM) has been described as a global epidemic1 and peripheral neuropathy its most common complication.2 The prevalence rates of diabetic neuropathy in a large study of Chinese subjects with type 1 and type 2 DM were approximately 22% and 35%, respectively.2 Comparable rates of diabetic neuropathy have been recently reported in Barbados, Libya, Qatar and South Korea.3–6 Approximately half of all individuals with DM develop a distal symmetrical and slowly progressive axonal polyneuropathy, with a stocking-and-glove distribution of sensory abnormalities.7,8

While the most common form of diabetic neuropathy is a central-peripheral distal axonopathy,9 which can be modeled in rodents by repeated systemic treatment with
the γ-diketone 2,5-hexanedione (2,5-HD), the molecular mechanisms underlying progressive axonal degeneration in DM are unknown. 2,5-HD reacts with the amino groups of axonal (and other tissue) proteins to form 2,5-dimethylpyrrole monomers that result in covalent cross-linking of derivatized proteins, including neurofilament and microtubule-associated proteins. This disrupts longitudinal axonal transport and eventually results in distal axonal degeneration of elongate large-diameter myelinated nerve fibers.

Since the serum of individuals with and without DM has been documented to contain 2-hexanone and 3-heptanone, both of which can undergo ω-1 oxidation to form the neurotoxic γ-diketones 2,5-HD and 3,6-heptanedione, respectively, that form amine pyrroles, we tested the novel hypothesis that elevated concentrations of γ-diketone pyrroles are present in diabetic urine.

Data reported here are consistent with this hypothesis and form the foundation for future studies to determine if there is a relationship between elevated levels of γ-diketone pyrroles and quantitative measures of stocking-and-glove neuropathy.

**RESEARCH DESIGN AND METHODS**

**Study participants**

The study included 534 participants: 267 persons with DM and 267 individuals serving as the control group. The participants were all from the baseline of the Shenzhen Aging-related Disorder Cohort established in Luohu district of Shenzhen City, Guangdong, China, which consists of 9411 older community members. The participants were all older individuals (aged ≥60 years) who responded to a questionnaire and were given a physical examination during the period from July 2017 to October 2018. Figure 1 shows the strict inclusion criteria of cases were: self-reported DM diagnosed by a physician plus evidence of the therapeutic use of insulin or other glucose-lowering agents, a fasting glucose level >7.0 mM and a glycated hemoglobin A1c (HbA1c) level of >6.5%. Inclusion criteria for healthy controls included: no self-reported diabetes plus no use of insulin or other glucose-lowering agents, a fasting blood level of <7.0 mM and an HbA1c level of <6.5%. Among the 9411 participants of the original cohort, 521 individuals fell in the group of those with DM and 4571 in the group of healthy controls. Among the participants with diabetes, those with missing samples (n=200) or missing information on cognitive functions (n=54) were excluded from the study. Case and control persons were matched at 1:1 by age and gender.

**Questionnaire and physical examination**

A general questionnaire administered by face-to-face interview was applied to all study individuals on the day of the physical examination. Information was collected on demographic characteristics (gender, birth date, occupation before retirement), lifestyle (active and passive smoking status), individual histories of chronic...
diseases (DM but not DM-type, hypertension and coronary disease) and medication history. The physical examination included measurements of height, weight, glucose level (coagulated blood) and HbA1c (EDTA-anticoagulated whole blood) of fasting venous blood, and routine urinalysis (urobilinogen and ketone bodies) of an early-morning sample. Body weight with light clothing and height without shoes were measured with an ultrasonic electronic height-and-weight scale (Omron HNH-219, Kyoto, Japan). The plasma glucose level of fasting venous blood was determined by a biochemistry autoanalyzer (Hitachi 7600-010, Hitachi, Tokyo, Japan). The HbA1c of fasting venous blood was determined by an HbA1c Analyzer (Premier Hb9210, Trinity Biotech, Bray, Ireland). Urinalysis used a urine analyzer (URIT-500B, URIT Medical Electronic Group, Shenzhen, China).

### Pyrrole analysis

Urine samples from the participants were collected in the early morning at the time of physical examination. Samples were stored at −20°C prior to use. Analysis of urinary pyrroles employed minor modifications of published methods.¹⁹⁻²¹ Pyrrole adducts were measured spectrophotometrically after reaction of 0.08 mL urine with 0.08 mL guanidine hydrochloride (70%) and 0.08 mL of Ehrlich’s reagent 3% 4-dimethylaminobenzaldehyde (DMBA) in the solution of 40% vol/vol methanolic 14% boron trifluoride and 60% vol/vol ethanol.²² Absorption values were measured at 526 nm with an automatic microplate reader (Infinite 1000, Tecan, Switzerland). Calculations were based on a standard curve prepared with different concentrations of 2,5-dimethylpyrrole.

### Data analysis

The Kolmogorov-Smirnov test was used to examine the distribution of variables. Continuous variables that distributed normally were expressed as a mean±SD. Non-normally distributed variables were presented as medians and interquartile range (IQR) and were compared between two groups by the Mann-Whitney U test. Categorical variables were reported as frequencies and proportions; these were compared between two groups using the χ² test and Fisher’s exact test if at least one cell had an expected count <5. Multiple linear regression analysis was used to determine the independent predictors of concentration of pyrrole adducts. Non-linearity in dose-response relationships between log-transformed diabetic indices (fasting blood glucose or HbA1c) and pyrrole adducts were assessed in the restricted cubic splines functions in linear models. Three knots (a term used in cubic spline functions) were set at the 5th, 50th and 95th percentiles of the distributions of pyrrole adducts. The covariates adjusted in the non-linearity in dose-response relationships included: smoking, body mass indices (BMI), coronary disease, hypertension. Participants were stratified into two gender subgroups in dose-response analyses. The statistical analyses were performed using SPSS (V.26.0; SPSS, Chicago, Illinois, USA) and SAS V.9.4 statistical software (SAS Institute, Cary, North Carolina, USA). P <0.05 was considered to be statistically significant.

### RESULTS

Characteristics of persons with and without DM are summarized in table 1. No significant difference was observed between cases and controls with respect to age, gender, urinary urobilinogen or occupational exposure to organic solvents prior to retirement. The DM group had significantly higher (p<0.01) levels of fasting blood glucose, HbA1c, urinary ketone bodies and pyrrole adducts than the control group. Of those with DM, the mean age of males (69.5 years, n=129) was higher (p=0.038) than females (68.5 years, n=138); no significant sex difference (p=0.80) was seen in fasting blood glucose levels (7.6 (4) mM and 7.5 (3) mM, respectively) or percentage (7.1 (2)) and of HbA1c (p=0.80).

The distribution of pyrrole adducts for both DM and controls is plotted in figure 2 and the key characteristics are summarized in table 2.

Of those with DM, mean levels of pyrrole adducts were higher (p<0.0001) in males (8.1 (4.5) μM) than females (6.7 (4.6) μM). The median concentration of pyrrole adducts was significantly higher and the maximum value was double in the DM group versus the control group (tables 1 and 2). Outliers (kurtosis) in the DM group exceeded those for controls (table 2). Higher skewness was also observed in the DM group (figure 2 and table 2).

The univariate linear regression model (table 3) showed pyrrole adduct levels were positively associated with age, DM, fasting blood glucose level, HbA1c and urinary ketone bodies. Pyrrole adduct concentration was also significantly associated with gender adduct concentration (p<0.0001), DM (p<0.0001), fasting blood glucose level (p<0.0001), HbA1c (p<0.0001) and urinary ketone bodies (p=0.088).

The multivariate linear regression model reflects that pyrrole adducts remained independently associated with DM, fasting blood glucose and HbA1c after adjustment for age, gender, smoking status, hypertension and coronary disease.

The dose-response relationships between pyrrole adducts and log-transformed diabetic indices (fasting blood glucose or HbA1c) were evaluated by restricted cubic spline analysis (figure 3). Strong overall association was observed for both fasting blood glucose (figure 3A, p for overall association <0.0001) and HbA1c (figure 3D, p for overall association <0.0001). Non-linear associations were found for both fasting blood glucose (p <0.0001) and HbA1c (p for non-linearity=0.0149). In the subgroup analysis, males and females showed an equivalent overall association in the dose-response relationships between pyrrole adducts and diabetic indices. Males showed higher nonlinearity (p=0.0005) than females (p=0.083) in the concentration-response relationship between pyrrole adducts and fasting blood glucose (figure 3B,C), while
females showed higher non-linearity ($p=0.0291$) than males ($p=0.1407$) in the relationship between pyrrole adducts and HbA1c (figure 3E,F).

**DISCUSSION**

We show that γ-diketone pyrrole adducts in urine samples of elderly Chinese individuals with DM are significantly elevated relative to healthy age-matched and gender-matched individuals. Healthy Chinese persons aged 21–50 years had urine pyrrole adduct levels approximately half those in serum. In rodents exposed to $n$-hexane, the highest concentration of pyrrole adducts was observed in kidney, followed by liver, brain, spinal cord, urine, sciatic nerve and serum.

We observed significant linear associations between urinary pyrrole adduct concentrations and diabetic indices (DM status, fasting blood glucose levels or HbA1c levels). We also found significant non-linear associations between urinary pyrrole adduct concentrations and two indices of DM (fasting blood glucose or HbA1c). Moreover, male persons showed a significant non-linear association between pyrrole adduct and fasting blood glucose levels, while females showed a non-linear association between pyrrole adducts and HbA1c. The generalizability of these findings is unknown.

**Table 1** Descriptive statistics of the study population

|                      | Healthy control | Diabetes mellitus | P value |
|----------------------|-----------------|-------------------|---------|
| Number of participants (F/M) | 267 (138/129)   | 267 (138/129)    | 1.000*  |
| Age of participants (years) | 68.3±4.9        | 68.3±4.9         | 1.000*  |
| Disease duration       | –               | 13.0±8.4         | –       |
| Taking glucose-lowering agent (N) | –               | 255             | –       |
| Taking insulin (N)     | –               | 42               | –       |
| Fasting blood glucose (mM) | 5.4 (0.7)      | 8.9 (2.5)        | <0.0001†|
| HbA1c (%)             | 5.9 (0.5)       | 7.8 (1.9)        | <0.0001†|
| Urinary ketone bodies (negative/weak positive/positive) | 267 (265/2/0) | 252/13/2        | 0.002*  |
| Urobilinogen (negative/weak positive/positive/strong positive) | 267 (265/0/1/1) | 267 (265/1/1/0) | 1.000*  |
| Occupational exposure to organic solvent (N/Y) | 267 (259/8) | 267 (261/6)     | 0.788   |
| Smoking (never/former and current) | 266 (204/62) | 267 (202/65)   | 0.839*  |
| BMI (<24 kg/m$^2$/>=24 kg/m$^2$/>24 kg/m$^2$) | 265 (136/107/22) | 266 (119/105/42) | 0.024*  |
| Hypertension (negative/positive) | 267 (122/145) | 267 (86/181)   | 0.002*  |
| Coronary disease (negative/positive) | 267 (156/11) | 263 (236/27)   | 0.007*  |
| Pyrrole adduct (μM)   | 5.9 (4.3)       | 7.5 (5.4)        | <0.0001†|

*Differences between groups were examined by the $\chi^2$ test.
†Differences between groups were examined by Mann-Whitney U test.

BMI, body mass index; HbA1c, glycated hemoglobin A1c.

**Figure 2** Violin plot of the distribution of pyrrole adducts. The thick black bar in the center represents the IQR and the white dot in the middle represents the median value. The thin black line extended from the thick black bar represents the upper (max) and lower (min) adjacent values of the data.

**Table 2** Concentration of pyrrole adduct in urine samples

|                      | Healthy control | Diabetes mellitus |
|----------------------|-----------------|------------------|
| Geometric mean (μM)  | 5.5             | 7.2              |
| Kurtosis             | 1.8             | 8.4              |
| Skewness             | 0.9             | 2.2              |
| Percentile (μM)      |                 |                  |
| 25                   | 3.9             | 5.1              |
| 50                   | 5.9             | 7.5              |
| 75                   | 8.2             | 10.5             |
| Maximum (μM)         | 20.5            | 42.6             |
While the three DM-associated classical ketone bodies (acetoacetate, β-hydroxybutyrate and their breakdown product acetone) are well known, a much larger number of ketones is found in human serum, including 3,6-heptanediol and 2,5-HD, respectively,15–17 repeated treatment with which results experimentally in axonal neuropathy.23 Noteworthy is that classical ketone bodies and 2-hexanone undergo γ-diketone oxidation (potentiated by γ-diketone pyrrole adducts of unstated origin of 0.91 nmol/mL, which corresponds to a concentration of 0.91 μM).31 This compares with the present findings of a median urinary pyrrole adduct level of 5.9 (4.3) μM for elderly healthy subjects, a result that might indicate pyrrole levels increase with age and correlate with the advance of sensory loss (notably vibration perception) with age.32 Importantly, age-matched subjects with DM in the present study had significantly higher (p=0.0001) urinary pyrrole levels and with greater variance, that is, 7.5 (5.4) μM.

Given our study involves an elderly residential population with no known current or recent exposure to exogenous 2,5-HD or its precursors, the present findings suggest the pyrrole-forming γ-diketone (or γ-diketones) arise from endogenous metabolism, in agreement with a previous study,27 with elevated levels associated with aging and in particular with diabetic dysmetabolism. This tentative conclusion should be strengthened by examination of a larger population over a wider age range, preferably in settings where airborne concentrations of n-hexane and 2-hexanone and serum/urine levels of γ-diketones are contemporaneously measured.

Demonstration that 2,5-HD induces central-peripheral distal axonopathy evolved from outbreaks of peripheral neuropathy among persons occupationally exposed to the solvent chemicals n-hexane or 2-hexanone in the presence or absence of 2-butanoate.28 Since n-hexane is an inexpensive and widely used commercial and industrial solvent,24 low levels are likely to be present in ambient air. Given that n-hexane is metabolized to 2,5-HD, an exogenous source might contribute to levels in human biofluids. One study found that 1.3% of 1200 normal Americans with no known occupational exposure to n-hexane had blood levels of the neurotoxic alkane.25 A Japanese study of 31 individuals with no known n-hexane exposure found low levels (<0.006 mg/L) of free 2,5-HD in urine.26 A third reported that healthy Italians without occupational exposure to n-hexane had detectable levels of 2,5-HD in urine (0.17–0.98 mg/L), only a minimal part of which was considered to have derived from exposure to hydrocarbon-polluted air.27 Subsequent study of urine samples from 123 healthy Italians recorded a 2,5-HD reference value of 0.795 mg/L for men and 0.627 mg/L for women.28 A fifth, very large investigation of healthy Chinese people (n=8235) with no occupational exposure to n-hexane or 2-hexanone, showed a median urine 2,5-HD concentration of 0.171 mg/L for males and 0.147 mg/L for females, with increasing 2,5-HD excretion with the advance of age.29 A sixth study of 227 Swedish persons randomly selected from the general population found that men had higher levels of 2,5-HD excretion than women (0.48 and 0.38 mg/L, respectively).30 In a seventh study, investigations of 208 male and female subjects aged 18–24 years revealed a median level of urinary pyrrole adducts of unstated origin of 0.91 nmol/mL, which corresponds to a concentration of 0.91 μM. This compares with the present findings of a median urinary pyrrole adduct level of 5.9 (4.3) μM for elderly healthy subjects, a result that might indicate pyrrole levels increase with age and correlate with the advance of sensory loss (notably vibration perception) with age.32 Importantly, age-matched subjects with DM in the present study had significantly higher (p=0.0001) urinary pyrrole levels and with greater variance, that is, 7.5 (5.4) μM.

### Table 3  Multiple linear regression analysis of independent predictors of pyrrole

|                        | B    | SE    | P value | Adjust R² |
|------------------------|------|-------|---------|-----------|
| **Univariate analysis**|      |       |         |           |
| Age                    | 0.021| 0.041 | 0.633   | -0.001    |
| Gender                 | -0.159| 0.395 | <0.0001 | 0.024     |
| Diabetes mellitus      | 0.252| 2.318 | <0.0001 | 0.062     |
| Fasting blood glucose  | 0.167| 0.071 | <0.0001 | 0.026     |
| HbA1c                  | 0.172| 0.117 | <0.0001 | 0.028     |
| Urinary ketone bodies  | 0.074| 0.974 | 0.088   | 0.004     |
| **Multivariate analysis**|    |       |         |           |
| Diabetes mellitus*     | 0.265| 0.392 | <0.0001 | 0.107     |
| Fasting blood glucose* | 0.169| 0.073 | <0.0001 | 0.067     |
| HbA1c*                 | 0.168| 0.120 | <0.0001 | 0.067     |

*Adjusted by age, gender, smoking status, BMI, hypertension, and coronary disease.
BMI, body mass index; HbA1c, glycated hemoglobin A1c.
Figure 3  The restricted cubic spline for associations between indices of diabetes and concentration of pyrrole adducts. Dose-response curve between log-transformed fasting blood glucose and concentration of pyrrole adducts in the overall study population (A), male only (B) and female only (C). Dose-response curve between log-transformed glycated hemoglobin A1c (HbA1c) and concentration of pyrrole adducts in the overall population (D), male only (E) and female only (F). The lines represent adjusted ORs (solid lines) and 95% CIs (long dashed lines). The reference values were set at 5th percentiles, and the knots were set at 20th, 5th, 50th and 95th percentiles of the log-transformed concentrations, respectively. Adjusted factors were consistent with the multivariate analysis of multiple linear regression analysis. FBG, fasting blood glucose.
The strengths and weaknesses of this study include the use of a sample nested in a very large and well-characterized cohort of elderly individuals. The pyrrole assay procedure employed a method that has been shown in experimental studies to reflect 2,5-HD concentrations in urine, although the DMBA method is semi-quantitative and might underestimate total pyrrole adducts. While the method cannot identify the specific amino targets of pyrrolization, the pyrrole-forming mechanism is a required step for induction of 2,5-HD axonopathy. Urinary urobilinogen could potentially interfere with the pyrrole assay but detectable levels were found in only 1.52% of study subjects in the total cohort of 9411 individuals, and there was no difference between subjects with and without DM. There was also no difference in the small number of persons in each group who reported prior occupational exposure to organic solvents, and chemicals and their metabolites arising in the workplace would have long before disappeared from the elderly retirees in this study. Follow-up studies are now needed to determine if urinary pyrrole adducts correlate with the results of quantitative sensory (vibration and temperature) and electrodiagnostic testing. Correlation of elevated pyrrole adduct levels with sensory loss would support (but not prove) an etiological role for \( \gamma \)-diketones in the induction of DM-associated stocking-and-glove neuropathy, which occurs more often in males than females. We found that levels of urinary 2,5-dimethylpyrrole adducts were somewhat higher in males than females with DM, as reported in healthy Chinese persons aged 31–50 years but not those aged 18–24 years.

Summary

We compared the \( \gamma \)-diketone pyrrole content of urine samples drawn from elderly Chinese individuals with and without DM. Urinary pyrrole levels were significantly elevated in individuals with DM (males > females). Both linear and non-linear relations were found between urinary pyroles and indicators of DM. This provides indirect evidence that diabetic dysmetabolism generates neurotoxic \( \gamma \)-diketones with potential to induce distal symmetrical polyneuropathy. This hypothesis can be tested by comparing urinary \( \gamma \)-diketone pyrrole levels with indices of sensory dysfunction.

Author affiliations

1Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
2Health Technology Assessment Center of Occupational Diseases, Shenzhen, Guangdong, China
3Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
4Poison Detection Center, Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, Guangdong, China
5Shenzhen Luohu Hospital for Traditional Chinese Medicine, Shenzhen, Guangdong, China
6Shenzhen Luohu Hospital for Traditional Chinese Medicine, Shenzhen, Guangdong, China
7Cognitive Impairment ward of Neurology, The 3rd Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong, China
8Shenzhen Luohu Center for Disease Control and Prevention, Shenzhen, Guangdong, China
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Competing interests None declared.

Patient consent for publication Not required.

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Data availability statement Data are available upon reasonable request. While the data sets generated and/or analyzed in the current study are not publicly available at this time, they are available to researchers on reasonable request. Specific ideas and proposals for potential collaboration are welcome and should be directed to the corresponding authors, primarily Professor Jianjun Liu (junli@126.com).

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ORCID iD

Peter Spencer http://orcid.org/0000-0003-3994-2639

REFERENCES

1 Feldman EL, Nave K-A, Jensen TS, et al. New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. Neuron 2017;93:1296–313.
2 Pan Q, Li Q, Deng W, et al. Prevalence of and risk factors for peripheral neuropathy in Chinese patients with diabetes: a multicenter cross-sectional study. Front Endocrinol 2018;9:517.
3 Adams OP, Herbert JR, Howitt C, et al. The prevalence of peripheral neuropathy severe enough to cause a loss of protective sensation in a population-based sample of people with known and newly detected diabetes in Barbados: a cross-sectional study. Diabet Med 2019;36:1629–36.
4 Garoushi S, Johnson MI, Tashani OA. A cross-sectional study to estimate the point prevalence of painful diabetic neuropathy in eastern Libya. BMC Public Health 2019;19:78.
5 Oh TJ, Kang S, Lee J-E, et al. Association between deterioration in muscle strength and peripheral neuropathy in people with diabetes. J Diabetes Complications 2019;33:598–601.
6 Ponirakis G, Elhadd T, Chinniyan S, et al. Prevalence and risk factors for painful diabetic neuropathy in secondary healthcare in Qatar. J Diabetes Investig 2019;10:1558–64.
7 Said G. Diabetic neuropathy—a review. Nat Clin Pract Neurol 2007;3:331–40.
8 Russell JW, Ziliiox LA. Diabetic neuropathies. Continuum 2014;20:1226–40.
9 Spencer PS, Schaumburg HH. Central-peripheral distal axonopathies—the pathology of dying-back polyneuropathies. In:
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Zimmerman HM, ed. Prog Neuropathol. New York: Grune & Stratton, 1976: 253–95.
10 Spencer PS, Schaumburg HH. Ultrastructural studies of the dying-back process. IV. Differential vulnerability of PNS and CNS fibers in experimental central-peripheral distal axonopathies. J Neuropath Exp Neurol 1977;36:300–20.
11 DeCaprio AP, O’Neill EA. Alterations in rat axonal cytoskeletal proteins induced by in vitro and in vivo 2,5-hexanedione exposure. Toxicol Appl Pharmacol 1985;78:235–47.
12 Genter MB, Szakál-Quin G, Anderson CW, et al. Evidence that pyrrole formation is a pathogenetic step in gamma-diketone neuropathy. Toxicol Appl Pharmacol 1987:37:351–62.
13 Redenbach DM, Richburg JH, Boekelheide K. Microtubules with altered assembly kinetics have a decreased rate of kinesin-based transport. Cell Motil Cytoskeleton 1994:27:79–87.
14 Heijink E, Scholten SW, Bolhuis PA, et al. Effects of 2,5-hexanedione on calpain-mediated degradation of human neurofilaments in vitro. Chem Biol Interact 2000;129:231–47.
15 Zlatkis A, Poole CF, Brazel CR, et al. Volatile metabolites in sera of normal and diabetic patients. J Chromatogr 1980;182:137–45.
16 Ichihara G, Amarnath V, Valentine HL, et al. Pyrrole adducts in globin and plasma of workers exposed to hexane. Int Arch Occup Environ Health 1999:72:873–81.
17 O’Donoghue JL, Krasavage WJ, DiVincenzo GD, et al. Further studies on ketone neurotoxicity and interactions. Toxicol Appl Pharmacol 1984:72:201–9.
18 Liu L, Liu W, Nie L, et al. Study design and baseline characteristics of Shenzhen ageing-related disorder cohort in China. BMJ Open 2020;10:e034317.
19 Yin H, Zhang C, Guo Y, et al. Biological exposure indices of pyrrole adducts in serum and urine for hazard assessment of n-hexane exposure. PLoS One 2014:9:e86108.
20 Yin H, Guo Y, Zeng T, et al. Correlation between levels of 2,5-hexanedione and pyrrole adducts in tissues of rats exposure to n-hexane for 5-days. PLoS One 2013:8:e76011.
21 DeCaprio AP, Strominger NL, Weber P. Neurotoxicity and protein binding of 2,5-hexanenedione in the hen. Toxicol Appl Pharmacol 1983:68:297–307.
22 Mattocks AP, Mota IN. Estimation of metabolites of pyrrolizidine alkaloids in animal tissues. Anal Biochem 1970:38:529–35.
23 Spencer PS, Schaumburg HH, Sabri M, et al. The enlarging view of hexacarbon neurotoxicity. Crit Rev Toxicol 1980:7:279–356.
24 Australian Government. n-Hexane: Sources of Emissions. National Pollutant Inventory. Available: http://npi.gov.au/resource/n-hexane-sources-emissions [Accessed 29 Jun 2020].
25 Chambers DM, Blount BC, McElprang DO, et al. Picogram measurement of volatile n-alkanes (n-hexane through n-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure. Anal Chem 2008;80:4666–74.
26 Sakai T, Araki T, Ushio K, et al. [Effect of hydrolysis conditions on the determination of urinary 2,5-hexanedione in workers exposed or not exposed to n-hexane]. Sangyo Igaku 1990;34:440–7. Japanese.
27 Perbellini L, Pezzoli G, Brugnone F, et al. Biochemical and physiological aspects of 2,5-hexanedione: endogenous or exogenous product? Int Arch Occup Environ Health 1993:65:49–52.
28 Bavazzano P, Apostoli P, Baldacci C, et al. Determination of urinary 2,5-hexanenedione in the general Italian population. Int Arch Occup Environ Health 1998:71:284–8.
29 Xing-Fu P, Ya-Ling Q, Wei Z, et al. Determination of total urinary 2,5-hexanedione in the Chinese general population. Environ Res 2016:150:645–50.
30 Persson B, Vretham M, Murgia N, et al. Urinary 2,5-hexanenedione excretion in cryptogenic polyneuropathy compared to the general Swedish population. J Occup Med Toxicol 2013:8:21.
31 Wang H, Wang Y, Zhou Z, et al. [Determination of normal reference value of pyrrole adducts in urine in young people in a university in Shandong, China]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2015;33:435–7.
32 Spencer PS, Ochoa J. The mammalian peripheral nervous system in old age. In: Johnson JE, ed. Aging and cell structure. New York, Plenum, 1981: 35–103.
33 Kobusch AB, Piao GL, du Souich P. Effects of acetone and methyl n-butyrate on hepatic mixed-function oxidase. Biochem Pharmacol 1989:38:3461–7.
34 Torres ME, Gonzalves LL, Bronze MR, et al. Alternative biomarkers of n-hexane exposure: characterization of aminoderived pyrroles and thiol-pyrrole conjugates in urine of rats exposed to 2,5-hexanenedione. Toxicol Lett 2014:224:54–63.
35 DeCaprio AP, Briggs RG, Jackowski SJ, et al. Comparative neurotoxicity and pyrrole-forming potential of 2,5-hexanenedione and perdeuterio-2,5-hexanenedione in the rat. Toxicol Appl Pharmacol 1989:92:75–85.
36 LoPachin RM, DeCaprio AP. gamma-Diketone neuropathy: axon atrophy and the role of cytoskeletal protein adduction. Toxicol Appl Pharmacol 2004:199:20–34.
37 The DCCT Research Group. Factors in development of diabetic neuropathy. Baseline analysis of neuropathy in feasibility phase of diabetes control and complications trial (DCCT). Diabetes 1988:37:476–81.
38 Aaberg ML, Burch DM, Hud ZR, et al. Gender differences in the onset of diabetic neuropathy. J Diabetes Complications 2008:22:83–7.
39 Booya F, Bandarian F, Larjani B, et al. Potential risk factors for diabetic neuropathy: a case control study. BMC Neurol 2005:5:24.