Pesticide Exposure and Diabetes

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Abbreviations

BMI	body mass index
CI	confidence interval
DDE	dichlorodiphenyltrichloroethylene
DDT	dichlorodiphenyltrichloroethane
HbA1c	glycohemoglobin
HDL	high-density lipoprotein
HOMA	homeostasis model assessment
NHANES	National Health and Nutrition Examination Survey

Glossary

Gestational diabetes   diabetes during pregnancy
Homeostasis model assessment (HOMA)   a measure of insulin resistance
Insulin resistance   results when insulin produced by the pancreas cannot get inside cells
Metabolic syndrome   co-occurrence of several common diabetes risk factors
Type 2 diabetes mellitus   adult onset diabetes
Units   lipids and glucose ($mmol\,l^{-1}$), insulin ($mU\,l^{-1}$), blood pressure (mmHg), pesticides ($ng\,g^{-1}$ lipid adjusted).

Introduction

Most pesticides can be classified as organochlorine pesticides, organophosphate pesticides, herbicides, pyrethroids pesticides, and carbamate insecticides. Many of the pesticides of interest are no longer used in developed countries, but they or their metabolites have persisted in the environment for many years. The link between pesticide use and type 2 diabetes mellitus has been difficult to establish. The research methods that have been used in this endeavor include animal studies, cross-sectional studies in humans, and a very limited number of longitudinal studies in humans. Type 2 diabetes is the result of problems in the production or use of insulin. Insulin is used to move glucose (blood sugar) into cells, for use as an energy source. When glucose cannot get into cells, too much of it remains in the blood causing diabetes. Insulin resistance results when insulin produced by the pancreas cannot get inside cells. More and more insulin is produced in response to this situation. Persons with insulin resistance are more likely to develop diabetes.

Common risk factors for type 2 diabetes include obesity, sedentary lifestyle, poor diet, family history of diabetes, race/ethnicity (in the United States, African Americans, Hispanics, and Native Americans; in the United Kingdom, South Asians), age, impaired fasting glucose, high blood pressure, low high-density lipoprotein (HDL) cholesterol, high triglycerides, and history of gestational diabetes. Separating the effect of pesticides from the effects of the common risk factors is an important task. In fact, pesticide or metabolite concentrations in blood have been shown to be associated with some of the common risk factors, raising the question as to which might cause diabetes. Another difficulty is that pesticides migrate with dioxins/furans, polychlorinated biphenyls, and other fat-soluble compounds, and it is difficult to determine which one(s) of the lipophilic compounds is really responsible for the association.

Associations of Pesticide Exposure with Common Diabetes Risk Factors

The National Health and Nutrition Examination Survey (NHANES) 1999–2002, conducted in the United States, has been used for a number of pertinent cross-sectional studies. One study of 721 nondiabetic participants focused on the association of pesticide metabolites in blood with metabolic syndrome. Metabolic syndrome is a term used to describe the co-occurrence of several common diabetes risk factors. These risk factors are more likely to occur together than they are to occur separately. The presence of three of the following five risk factors classifies a person as having metabolic syndrome:

1. Obesity, defined as waist circumference $>102\,cm$ in men and $>88\,cm$ in women.
2. Serum triglycerides $\geq 1.7\,mmol\,l^{-1}$.
3. Serum HDL cholesterol $<1.1\,mmol\,l^{-1}$ in men or $<1.5\,mmol\,l^{-1}$ in women.
4. Blood pressure $\geq 130\,mmHg$ systolic or $\geq 85\,mmHg$ diastolic, or taking antihypertensive medication.
5. Impaired fasting glucose, defined as serum glucose $\geq 5.6\,mmol\,l^{-1}$ and $<7.0\,mmol\,l^{-1}$.

Metabolic syndrome is considered a prediabetes state as is impaired fasting glucose by itself.

Four metabolites of pesticides were studied using the NHANES 1999–2002: beta-hexachlorocyclohexane, a metabolite of lindane; oxychlordane and *trans*-nonachlor, both metabolites of chlordane; and p,p'-dichlorodiphenyltrichloroethylene (p,p'-DDE), a metabolite of

dichlorodiphenyltrichloroethane (DDT). All four serum metabolites were lipid adjusted. Logistic regressions were adjusted for age, gender, race, poverty income ratio, smoking, serum cotinine, alcohol consumption, and exercise. Serum beta-hexachlorocyclohexane $\geq$ 75th percentile was significantly associated with metabolic syndrome (odds ratio 3.1, 95% confidence interval (CI) 1.4–6.5) and impaired fasting glucose (odds ratio 4.0, 95% CI 1.1–14.3) when compared to nondetectable beta-hexachlorocyclohexane. In addition, p,p'-DDE $\geq$ 75th percentile was associated with impaired fasting glucose (odds ratio 6.6, 95% CI 1.8–24.3). Neither oxychlordane nor *trans*-nonachlor was associated with metabolic syndrome or impaired fasting glucose. None of the four organochlorine pesticide metabolites was associated with obesity.

The Agricultural Health Study is a large study of farmers and other licensed pesticide applicators, and their families, in Iowa and North Carolina (USA). A total of 52 395 farmers and commercial applicators, and 32 171 spouses were enrolled between 1993 and 1997. Of the spouses, 61% completed a female and family health questionnaire. Following exclusions, 11 273 pregnancies were identified that were suitable for a study of pesticide exposure and gestational diabetes. Gestational diabetes is considered an early stage in the progression to type 2 diabetes and risk factors are similar for both. Pesticide exposure during the first trimester of pregnancy was classified as none, indirect (planting, pruning, weeding, picking, or harvesting), residential (applying to home or garden), and agricultural (mixing or applying pesticides or repairing pesticide-related equipment). Compared to no exposure, agricultural exposure during the first trimester was significantly associated with gestational diabetes (odds ratio 2.2, 95% CI 1.5–3.3) in a logistic regression adjusted for body mass index (BMI) at enrollment, mother's age at delivery, parity (previous live births and stillbirths), race, and state (Iowa versus North Carolina). Indirect exposure and residential exposure were not significantly different from no exposure. A total of 337 women were categorized as agriculturally exposed. Among these women, gestational diabetes was significantly associated with the reporting of ever-use of the herbicides 2,4,5-T, 2,4,5-TP/silvex, atrazine, and butylate; the organophosphate insecticides diazinon and phorate; and the carbamate carbofuran in logistic regressions adjusted for the above-mentioned covariates and the five most common pesticides reported by women in the study (glyphosate; carbaryl; malathion; 2,4-D; and diazinon).

Associations of Pesticide Exposure with Insulin Resistance

The NHANES 1999–2002 has been used to study the association of pesticide metabolites and insulin resistance in 749 nondiabetic participants. Insulin resistance precedes the development of diabetes, but the degree of insulin resistance associated with diabetes varies from person to person. A simple measure of insulin resistance that is based on fasting insulin and fasting glucose is the homeostasis model assessment (HOMA). HOMA is calculated by the following equation: (insulin $(mU\,l^{-1}) \times$ glucose (mmol $l^{-1})/22.5$). Beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and p,p'-DDE were again evaluated. All four serum metabolites were lipid adjusted. Logistic regressions were adjusted for age, gender, race, poverty income ratio, BMI, waist circumference, smoking, serum cotinine, alcohol consumption, and exercise. HOMA $\geq$ 90th percentile ($\geq$ 5.06), a high value, was associated with oxychlordane (odds ratio 8.7, 95% CI 2.3–33.3) and *trans*-nonachlor (odds ratio 5.4, 95% CI 1.3–23.1) $\geq$ 75th percentile compared to nondetectable oxychlordane and *trans*-nonachlor, respectively. Beta-hexachlorocyclohexane and p,p'-DDE were not associated with elevated HOMA, even though, as described earlier, they were associated with impaired fasting glucose. The organochlorine pesticide metabolites, taken together, showed a nonsignificant interaction with waist circumference. Elevated HOMA appeared to be more likely in the fourth quartile of organochlorine pesticide metabolites when waist circumference was greater than or equal the second tertile. This finding suggests pesticides are a more important risk factor for obese persons.

Cross-Sectional Associations of Pesticide Exposure with Type 2 Diabetes

Two additional studies have been done using the NHANES 1999–2002, this time to evaluate the association of pesticides and pesticide metabolites with type 2 diabetes. In these studies, both diagnosed and undiagnosed diabetes were evaluated. Undiagnosed diabetes is presumed when fasting glucose is $\geq$ 7.0 mmol l^{-1}, nonfasting glucose is $\geq$ 11.1 mmol l^{-1}, or nonfasting glycohemoglobin (HbA1c) is > 6.1%. In one study, 1721 participants, including 179 persons with diabetes, were assessed for the association of beta-hexachlorocyclohexane, oxychlordane, *trans*-nonachlor, and p,p'-DDE with type 2 diabetes. All four serum metabolites were lipid adjusted. Logistic regressions were adjusted for age, gender, race, poverty income ratio, BMI, and waist circumference. All four pesticide metabolites were significantly associated with type 2 diabetes, with odds ratios ranging from 2.9 (95% CI 1.5–5.6) for p,p'-DDE $\geq$ 75th percentile to 8.0 (95% CI 2.6–24.8) for *trans*-nonachlor $\geq$ 75th percentile, both compared to nondetectable levels of the respective metabolites.

In a separate study, p,p'-DDT was evaluated in 2163 participants of the NHANES 1999–2002. Logistic

regressions were adjusted for age, gender, race, country of birth, education, poverty income ratio, BMI, waist circumference, and physical activity. Both diagnosed diabetes (odds ratio 2.14, 95% CI 1.03–4.46) and undiagnosed diabetes (HbA1c >6.1%) (odds ratio 2.58, 95% CI 1.25–5.33) were associated with p,p'-DDT >26.6 ng g^{-1} lipid adjusted when compared to p,p'-DDT ≤20.7 ng g^{-1} lipid adjusted. Persons who have diabetes have been hypothesized to accumulate pesticides, and pesticide metabolites, due to impaired elimination of the compounds from the body. When participants with poor liver and kidney functions were excluded from the analysis, p,p'-DDT was still significantly associated with type 2 diabetes. To test whether or not p,p'-DDT accumulates in persons who have diabetes, the serum p,p'-DDT concentration was regressed with the number of years a person has had diabetes. The beta (slope) of the regression line was not significantly different from zero, indicating there was no correlation.

Three studies of pesticides and pesticide metabolites have been conducted in Sweden. The first assessed 205 Swedish women, including 7 with diabetes, for hexachlorobenzene, beta-hexachlorocyclohexane, oxychlordane, trans-nonachlor and p,p'-DDE. All five compounds were lipid adjusted. Geometric means were adjusted for region within Sweden, age, BMI, and weight change. The participants with diabetes had significantly higher hexachlorobenzene (85 ng g^{-1} lipid adjusted, 95% CI 66–109) than those without diabetes (60 ng g^{-1} lipid adjusted, 95% CI 58–63). None of the other four pesticide metabolites tested had significantly different concentrations between participants with diabetes and those who did not have diabetes. However, all five compounds were significantly correlated with one another ($r = .48–.89$). In the second study conducted in Sweden, 196 men (including 12 with diabetes) and 184 women (including 10 with diabetes) were evaluated. In an unadjusted logistic regression, an increase of 100 ng g^{-1} lipid adjusted p,p'-DDE had an odds ratio of 1.05 (95% CI 1.01–1.09) for diabetes. Adjustment for gender, current age, and BMI at 25 years of age did not substantially change this result. In the third Swedish study, 543 women (including 15 with type 2 diabetes) were assessed. In unadjusted logistic regression, an increase of 100 ng g^{-1} lipid adjusted p,p'-DDE had an odds ratio of 1.3 (95% CI 1.1–1.5) for diabetes. Inclusion of age in the model did not change the association.

In New York (USA), 352 Native Americans (Mohawk) were assessed for DDE, hexachlorobenzene, and mirex. Twenty percent of the participants had diabetes. Logistic regressions were adjusted for gender, age, BMI, and lifetime smoking status. The highest tertile of lipid-adjusted hexachlorobenzene was significantly associated (odds ratio 6.8, 95% CI 2.3–20.3) with diabetes compared to the lowest tertile, as was the highest tertile of lipid-adjusted DDE (odds ratio 6.2, 95% CI 1.8–21.9) compared to the lowest tertile of DDE. Mirex was not associated with diabetes.

In Australia, a cohort of outdoor workers, a portion of which worked in an insecticide application program, was followed for mortality through 1996. Survivors were sent questionnaires, with 55% of 1533 known surviving participants having returned completed questionnaires. Subjects reporting occupational uses of herbicides were significantly more likely to have diabetes (odds ratio 2.26, 95% CI 1.15–4.43) in a logistic regression adjusted for age and smoking.

Longitudinal Studies of Pesticide Exposure and Type 2 Diabetes

A quasi-longitudinal study of US workers occupationally exposed to pesticides was conducted between 1971 and 1978. A total of 73% of the 3669 participants enrolled in 1971–73 were followed till 1977–78. Surviving participants completed a questionnaire about diseases suffered between 1971 and 1977, which they did not have before 1971. Hence, it is possible that a participant had diabetes at enrollment when the laboratory work was done, but only for up to a few years. The age-adjusted geometric mean serum DDT + DDE concentration was 29% higher in persons having developed diabetes between 1971 and 1977 than persons not having diabetes. Although serum DDT + DDE concentrations were not lipid adjusted in this study, total cholesterol concentration was not significantly different between the diabetes group and the control group.

The Agricultural Health Study has been used for a longitudinal study of incident diabetes among 31 787 licensed pesticide applicators. Participants enrolled at baseline (1993–97) were contacted 5 years later for a follow-up interview (1999–2003). Persons having diabetes at baseline were excluded, and persons developing diabetes, by the follow-up interview, plus those without diabetes were evaluated for exposure to 49 pesticides. Ever-use of the organochlorines chlordane (odds ratio 1.16, 95% CI 1.01–1.34) and heptachlor (odds ratio 1.20, 95% CI 1.01–1.43); of organophosphates coumaphos (odds ratio 1.26, 95% CI 1.03–1.55), phorate (odds ratio 1.22, 95% CI 1.06–1.42), terbufos (odds ratio 1.17, 95% CI 1.02–1.35), and trichlorfon (odds ratio 1.85, 95% CI 1.03–3.33); and of the herbicides alachlor (odds ratio 1.14, 95% CI 1.00–1.30) and cyanazine (odds ratio 1.27, 95% CI 1.09–1.47) was associated with incident diabetes in logistic regressions adjusted for age, state (Iowa versus North Carolina), and BMI. In addition, a dose–response relationship with cumulative days of use was found for the organochlorine heptachlor, the organophosphates chlorpyrifos, diazinon, and trichlorfon; and the herbicides

alachlor and cyanazine. A limitation of this study is that incident diabetes was determined by self-report.

Longitudinal studies of pesticide exposure and diabetes mortality in Australia and America, although of interest, have to be viewed with reservations. Type 2 diabetes is a risk factor for coronary heart disease, and therefore persons having diabetes are more likely to die of heart disease and not diabetes. The focus of this article is on diagnoses of diabetes as the outcome of interest to avoid confusing diabetes with its complications.

Proposed Mechanisms by Which Pesticide Exposure Would Cause Type 2 Diabetes

The precise mechanisms through which pesticides induce diabetes remain unclear but several likely mechanisms have been proposed for organophosphates. Organophosphates can affect glucose metabolism by blocking cholinesterase activity, oxidative stress, nitrosative stress, physiological stress, adrenal stimulation, and inhibition of paraoxanse.

Cholinesterase is an enzyme that breaks down the neurotransmitter acetylcholine. Organophosphates inhibit cholinesterase, resulting in the accumulation of a large amount of acetylcholine. As acetylcholine accumulates in a poisoning victim, he will suffer from uncontrolled muscle spasms, tremors, and even convulsions. To meet the increased energy demands resulting from the muscle spasms and tremors, the body will release glucose stored in the liver, increasing the blood glucose level. With chronic organophosphate exposure, the glucose level will remain persistently elevated, ultimately contributing to the development of diabetes.

Over recent years, the role of oxidative stress in damaging DNA, cellular proteins, and cell membranes has become well established. Organophosphates are believed to mediate cellular oxidation through the release of several oxidizing compounds, the most important of which is thought to be peroxynitrite. In an effort to combat the damaging effects of such oxidizing compounds, the body increases serum glucose levels, which, in turn, increases the production of the potent antioxidant glutathione. When organophosphate exposure is brief, this compensatory increase in glucose is beneficial, but with chronic exposure, diabetes can ultimately develop.

Organophosphates are believed to increase reactive nitrogen species. When under oxidative stress, these reactive nitrogen species can lead to pancreatitis and may directly damage pancreatic β-cells through the formation of cytokines, ultimately contributing to diabetes.

Inhibition of acetylcholine receptor sites in the adrenal medulla by organophosphates could lead to the increased production of adrenaline. Adrenaline accelerates glycogen breakdown in the liver and skeletal muscles, thereby increasing serum glucose. In addition, adrenaline increases free fatty acid levels, which decreases the efficacy of insulin.

As might be anticipated, organophosphate exposure induces a classic stress response by the body. Following exposure, the sympathetic nervous system is stimulated, which, in turn, leads to the release of glucagon, growth hormone, and catecholamines. Glucagon, growth hormone, and catecholamines promote glycogenolysis, gluconeogenesis, and insulin resistance, which rapidly lead to hyperglycemia. The release of cortisol from the adrenal cortex is a typical stress reaction. Cortisol induces glyconeogensis as well as insulin resistance by decreasing the ability of skeletal muscle to uptake glucose. Stress also leads to the release of inflammatory cytokines from immune cells. Inflammatory cytokines increase glycogen breakdown in the liver, thereby increasing blood glucose levels. In addition, cytokines decrease the ability of skeletal muscles to uptake glucose, thereby increasing insulin resistance. In the short term, such a stress response can protect brain cells by providing needed glucose to overcome the specific stress at hand. With chronic exposure, however, such a situation can lead to diabetes.

Paraoxonase is an enzyme that is generally believed to be involved in detoxification of reactive oxygen species. This enzyme has been found to be decreased in individuals with both type I and type II diabetes, as well as in those suffering from organophosphate poisoning. By decreasing oxidative stress, this enzyme lowers serum glucose levels. As paraoxonase levels fall, oxidative stress appears to increase, as do serum glucose levels.

Although the precise mechanisms by which organophosphates induce diabetes in humans are not fully elucidated, inhibition of cholinesterase activity, oxidative stress, nitrosative stress, physiological stress, adrenal stimulation, and inhibition of paraoxanse appear to be major contributors. Such knowledge seems particularly important when one considers the ubiquitous nature of organophosphate pesticides in the environment. In 1997, an estimated 32 million kg of organophosphates were used in the United States. Although much of this was used agriculturally, organophosphates are still commonly used in household insecticides.

Further Reading

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