Insulin sensitivity and serum TCDD in Air Force veterans occupationally exposed to herbicides during the Vietnam War

Philip Kern[1], Sufyan Said[1], William Jackson Jr[2], Joel Michalek[2]

[1]University of Arkansas for Medical Sciences, Little Rock
[2]Air Force Research Laboratory, San Antonio

Introduction

Between 1961 and 1971, the United States Air Force sprayed 12 million gallons of the defoliant "Agent Orange" on 3.6 million acres of Vietnam. Agent Orange was a 1:1 mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid)[1], and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was a contaminant of the defoliant, from less than 0.05 to almost 50 parts per million. Numerous Vietnam veterans were exposed to TCDD when Agent Orange and other TCDD-contaminated herbicides were sprayed in large quantities in Vietnam[2] and TCDD has been found at many toxic waste disposal sites in the United States. Some of the highest exposure to TCDD occurred in members of Operation Ranch Hand, the Air Force unit responsible for spraying herbicides from fix-wing aircraft in Vietnam. The Air Force Health Study (AFHS), an epidemiological study of Ranch Hand veterans, was launched in 1980 to address veteran concerns regarding Agent Orange exposure.

A link between TCDD and diabetes has been demonstrated in several studies. Among the Ranch Hand veterans with high blood levels of TCDD, there was a significant increase in the prevalence of diabetes and a decrease in the age at which diabetes was diagnosed[3]. In a study from Seveso, Italy, where 45,000 people had varying levels of exposure to TCDD, there were significant increases in mortality from coronary artery disease and diabetes[4]. Several studies have demonstrated a relationship between blood TCDD levels and hyperinsulinemia[5,6]. The data suggest that non-diabetic individuals exposed to TCDD have an increased risk of insulin-resistance, being able to maintain normal blood glucose levels but only because of very high concentrations of insulin. As a result of available

evidence, public policy decisions have been made, such as a decision by the Veterans Administration that diabetes is a service-connected condition in Agent Orange-exposed Vietnam veterans. Here we study the relation between TCDD insulin sensitivity in a subset of AFHS participants.

Materials and Methods

The AFHS is an ongoing prospective epidemiological investigation that seeks to determine if veterans of Operation Ranch Hand, the unit responsible for aerially spraying herbicides during the Vietnam War, have experienced adverse health that can be attributed to exposure to herbicides or their TCDD contaminant. A Comparison group of other Air Force veterans who served in Southeast Asia during the same period that the Ranch Hand unit was active but who were not involved with spraying herbicides serves as a reference. In the full Air Force Health Study, Comparison veterans were matched to Ranch Hands veterans with respect to age, race and military occupation.

All AFHS study subjects are male, and six periodic physical examinations were performed in 1982, 1985, 1987, 1992, 1997 and 2002. Participation was voluntary and informed consent was given at the examination sites. The study includes assessments of the health, mortality experience and reproductive outcomes. The current sub-study was conceived because previous investigations suggested a relationship between TCDD levels and diabetes[6].

Blood from willing AFHS participants was collected and TCDD was measured in parts per trillion (ppt) serum lipid at the Centers for Disease Control and Prevention. The serum TCDD measurements were done with high-resolution gas chromatography/high resolution mass spectrometry. The between assay coefficient of variation at three different concentrations of TCDD ranged from 9.4% to 15.5%. Most TCDD measurements were made in serum collected at the 1987 examination. For those veterans whose TCDD level was not obtained in 1987, measurements were made in 1992 or 1997 and extrapolated to 1987 using a first-order kinetics model with a constant half-life of 7.6 years.

During the 1997 AFHS physical examination, a 75-gram oral glucose tolerance test was performed. For this insulin sensitivity sub-study, we limited our selection of subjects to those without diabetes or impaired glucose tolerance, based on a standard 75-gram oral glucose tolerance test (fasting glucose <110 mg/dl, 2 hour post-prandial glucose <140 mg/dl). We restricted this sub-study to non-

diabetic veterans because earlier analyses found mean post-prandial insulin increased in non-diabetic Ranch Hand veterans with high TCDD exposure[7]. We designed this sub-study to match (one-to-one) 30 Ranch Hand subjects with high TCDD exposure to 30 comparison subjects. Each pair (comprised of one Ranch Hand and one Comparison) was matched on age (within 5 years), body mass index (BMI) (within 2 kg/m^2), race (Black, non-black), and a family history of diabetes in first order relatives (yes, no) as reported on questionnaires administered at the 1997 physical examination. Of the eligible 567 Ranch Hand veterans, we selected 71 who had four TCDD measurements from serum collected in 1982, 1987, 1992 and 1997, each of which was greater than 10 ppt, a value we use as an approximate threshold for background exposure. Of the 815 eligible Comparison veterans, we excluded 13 who had a measured TCDD greater than 10 ppt. Six hundred sixty two of the remaining 802 eligible Comparisons could not be matched to any of the 71 Ranch Hand veterans, leaving 71 matched sets. From these 71 matched sets, we invited 30 Ranch Hand veterans and 30 of their matched Comparisons, to participate in the matched-pair sub-study. Thus, the Ranch Hand subjects had had consistently high TCDD levels since at least 1982, and the Comparison group had low TCDD levels.

Prior to being invited for insulin sensitivity testing, the paired veterans were interviewed by telephone, and fasting laboratory testing was performed. The interview was focused on determining any concurrent medical conditions, medications, and weight. Exclusion criteria included (a) a weight gain or loss of more than 5% since the 1997 physical examination, (b) the occurrence of any chronic or acute illness that may have affected insulin sensitivity (including inflammatory conditions such as rheumatoid arthritis, and recent acute medical event, such as myocardial infarction), (c) taking medications likely to affect insulin sensitivity (such as corticosteroids), and (d) the occurrence of liver abnormalities, renal dysfunction, anemia, or electrolyte disturbances.

Sixty veterans (comprising the 30 matched pairs) traveled to General Clinical Research Center at the University of Arkansas for Medical Sciences/Central Arkansas Veterans HealthCare System for insulin sensitivity testing. Upon arrival, consent forms were signed and medical history was confirmed by personal interview. Subjects spent a restful evening, stayed overnight, and were awakened at 0700 for insulin sensitivity testing.

The measurement of *in vivo* insulin sensitivity was performed in the fasting state using the minimal model analysis of the frequently sampled intravenous glucose tolerance test (FSIVGTT). We used the classic tolbutamide-modified test, which has been validated against the euglycemic clamp in humans. In brief, catheters were placed for glucose injection, and for blood sampling. Four basal blood samples were obtained and the patient was given an IV glucose bolus (11.4 g/m^2) at time 0. At 20 min after the glucose injection, patients were given an injection of tolbutamide (125 mg/m^2) again followed by frequent blood sampling, according to the standard protocol. Together, 4 basal and 27 post-glucose blood samples were taken, the last one at 240 min. Glucose was measured using glucose oxidase method in a glucose analyzer and insulin was measured using radioimmunoassay. These measurements were performed in the Endocrinology Laboratory of the Indiana University School of Medicine (Indianapolis, IN). The insulin sensitivity index (S_I) was calculated using the MINMOD program, and expressed in units of $min^{-1}/(\mu U/ml)$. The acute insulin response to glucose (AIR_G) was also determined as the area under the insulin curve during the first 2-10 minutes following the glucose injection (mg-min/dl). A Disposition Index was computed as the product of AIR_G and S_I. Because one Comparison had an S_I that was indeterminate secondary to poor insulin secretion, we analyzed data from 29 matched pairs.

In some of these veterans, measurements were also made of circulating inflammatory cytokines that are known to be associated with insulin resistance. Fasting plasma levels of TNFα (pg/ml) and adiponectin (μg/ml) were measured in each member of each pair. The measurement of adiponectin protein employed a radioimmunoassay (Linco Research, St. Charles, MO). This assay demonstrates a 4.3% intraassay variation, and a 7.1% interassay variation. TNFα was measured using ELISA assays (R&D Systems Minneapolis, MN).

We analyzed S_I, AIR_G, and the Disposition Index, in log units. We analyzed TNFα in log units and adiponectin, in original units, on a subset of 40 veterans in 20 matched pairs with complete data for these two variables. For each outcome variable, we tested the hypothesis of equal group means with a paired t-test and regressed within-pair differences of the dependent variable on within-pair differences of TCDD in log units (base 2). Differences of variables in log units were expressed as the logarithm of the ratio of the Ranch Hand value to the Comparison value. The result of a test of hypothesis was called significant if $p \leq 0.05$ and borderline significant if $0.05 < p \leq 0.10$.

Results

Twenty-nine matched pairs of subjects successfully completed insulin sensitivity testing using the FSIVGTT with minimal model analysis. There were no significant differences in mean age, BMI, percentage with a family history of diabetes, or mean hemoglobin A_1C, triglycerides, cholesterol, HDL cholesterol, fasting glucose, or fasting insulin. Even though the basis of the matching included data on BMI, glucose tolerance, and family history from 1997, the pairs were still well matched at the time of insulin sensitivity testing (between December 1999 and March 2001). There were large differences in serum TCDD levels between the groups by design. The 29 selected Ranch Hand veterans contained fewer individuals who were officers while serving in the Air Force in Vietnam.

There were no significant differences in mean insulin sensitivity (S_I), insulin secretion (AIR_G), or Disposition Index between Ranch Hand and Comparison veterans, nor were there differences in mean blood adiponectin or TNFα (data not shown).

Although the Ranch Hand and comparison groups were discordant for blood TCDD levels, the magnitude of the difference in TCDD between pairs varied, based on the background exposure to TCDD in the comparisons, possible differences in the initial dose, and variation in the decrease in TCDD in the Ranch Hands since the time of original Agent Orange exposure. To determine whether the difference in TCDD levels between individuals in a pair was related to the difference in insulin sensitivity, we performed additional outcome analyses. Within-pair differences of measures of insulin sensitivity (Ranch Hand minus Comparison) were regressed on within-pair differences of TCDD levels in log (base 2) units (Table 1). The slope relating within-pair differences of S_I to within-pair differences on TCDD was negative and reached significance ($p=0.01$). Stated differently, pairs with the greatest difference in TCDD levels demonstrated the largest decrease in S_I, and hence the largest amount of insulin resistance. Using this analysis, we attempted to examine the magnitude of the effect of blood TCDD level on insulin resistance. Our regression model predicted a 10% decrease in S_I for every 18-fold difference in TCDD levels between a Ranch Hand and his matched Comparison. In addition, using the same analysis, there was borderline significance for adiponectin ($p=0.09$), and TNFα ($p=0.10$), and the slopes were positive for TNFα, and negative for adiponectin, which is consistent with the known actions of these cytokines to respectively promote or resist insulin resistance.

Table 1. **Regression model results***

Dependent variable	Number of paired differences	Intercept (SE)	Slope (SE)	P value (H_o: slope=0)
S_I	29	1.42† (0.56)	-0.368 (0.135)	0.01
AIR_G	29	-0.62 (0.69)	0.174 (0.167)	0.31
Disposition Index	**29**	0.80 (0.84)	-0.194 (0.204)	0.35
TNFα	20	-0.65 (0.40)	0.172 (0.100)	0.10
Adiponectin	20	5.35 (3.50)	-1.541 (0.871)	0.09

* The dependent variables were within-pair differences (Ranch Hand - Comparison) and the independent variable was the within-pair difference of log-transformed TCDD, given by $\log_2(TCDD_{RH}/TCDD_C)$. The slopes estimate the change in the dependent variable for each doubling of serum TCDD. S_I, AIR_G, the Disposition Index and TNFα were log-transformed prior to analysis

The regression line relating within-pair differences on S_I and within pair differences of the logarithm (base 2) of TCDD levels is shown in Figure 1.

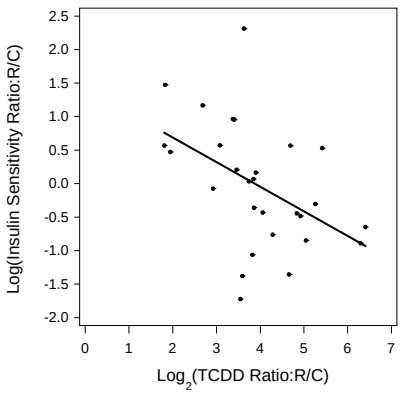

Figure 1. **Within-pair differences in SI versus within-pair differences in TCDD. Based on 29 matched pairs of Ranch Hand and Comparison subjects, the difference in log SI (Ranch Hand – Comparison) was regressed against the log2 difference in TCDD.**

Therefore, these data do not demonstrate differences between groups using paired t-tests. However, the within-pair differences between subjects were consistent with a subtle effect of blood TCDD level to promote insulin resistance.

Discussion

Because of the exposure of many Vietnam veterans and others to Agent Orange and other herbicides, there has been a great deal of research of potential long-term consequences. Much attention has been focused on TCDD, the most toxic of the dioxin compounds and a contaminant of Agent Orange, which has also been found at numerous toxic waste sites. Previous studies have identified a statistical link between TCDD levels and diabetes or insulin resistance. There are a number of possible mechanisms for TCDD-mediated insulin resistance. A number of studies in vitro have demonstrated an increase in cellular expression of TNFα after exposure to TCDD. Elevated TNFα expression from adipose tissue is linked to the development of insulin resistance, and TCDD is concentrated in adipose tissue, raising the possibility that TCDD exposure contributes to the adipose tissue-mediated proinflammatory condition associated with the metabolic syndrome.

The Ranch Hand study is a long-standing prospective epidemiologic study that is unique because of the extensive characterization of the participants, and the measurement of blood TCDD levels in both the index and control groups. This study was intended to determine whether Vietnam veterans who were matched according to age, race, BMI, and family history of diabetes, and who differed primarily on serum levels of TCDD, demonstrated differential degrees of insulin resistance and related parameters. We found no significant mean differences in S_I between the Ranch Hand and Comparison groups. This lack of a difference between groups is consistent with either no effect of TCDD on insulin sensitivity, or with a subtle effect that cannot be detected using this method of analysis. To determine whether there was a subtle effect of TCDD on insulin resistance, we examined within-pair differences on S_I and found that insulin sensitivity decreased significantly with regard to within pair differences on TCDD in log units. These changes in insulin sensitivity were accompanied by a trend towards changes in the plasma level of cytokines TNFα and adiponectin that would be consistent with a TCDD-mediated worsening of insulin sensitivity.

In conclusion, we measured insulin sensitivity using a frequently sampled intravenous glucose tolerance test (S_I) in a study of 29 matched pairs of Ranch Hand and Comparison veterans who attended the 1997 examination. We found no significant difference between Comparison and Ranch Hand veterans in mean S_I. However, within-pair differences on S_I decreased significantly with regard to

within-pair differences on TCDD in the adverse direction. The same pattern of an adverse trend with TCDD was found for TNF_{α} and adiponectin. Although the biological meaning of these patterns is difficult to resolve, these data suggest that prior TCDD exposure had a small effect that may promote insulin resistance, and lead to increased susceptibility to type 2 diabetes.

References

1. Young AL, Calcagni JA, Thalken CE, Tremblay JW. *The toxicology, environmental fate, and human risk of herbicide orange and its associated dioxin.* Brooks AFB TX: USAF OEHL (Technical Report TR-78-92) 1978.
2. Schecter A, Dai LC, Thuy LT, Quynh HT, Minh DQ, Cau HD, Phiet PH, Phuong NTN, Constable JD, Baughman R, Papke O, Ryan JJ, Furst P, Raisanen S. *Am J Public Health* 1995;85:516-22
3. Henriksen GL, Ketchum NS, Michalek JE, Swaby JA. *Epidemiology* 1997;8:252-258
4. Pesatori AC, Zocchetti C, Guercilena S, Consonni D, Turrini D, Bertazzi PA. *Occup Environ Med* 1998; 55(5):126-31
5. Cranmer M, Louie S, Kennedy RH, Kern PA, Fonseca VA. *Toxicol Sci* 2000;56(2):431-436
6. Institute of Medicine. *Veterans and Agent Orange: Herbicide/Dioxin Exposure and Type 2 Diabetes.* Washington DC: National Academies Press 2000.
7. Michalek JE, Akhtar FZ, Kiel J. *J Clin Endocrinol Metab* 1999;84:1540-1543