# INSULIN SENSITIVITY AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN US AIR FORCE VETERANS OF THE VIETNAM WAR

Philip A. Kern[1], Sufyan Said[1], William G. Jackson Jr[2], and Joel E. Michalek[2]

1. The Department of Medicine, Division of Endocrinology, University of Arkansas for Medical Sciences, and the Central Arkansas Veterans Healthcare System, Little Rock, Arkansas, USA.
2. Air Force Research Laboratory, Brooks City-Base, Texas, USA.

## Introduction

Previous studies have suggested a link between toxic exposures and an increased risk of developing diabetes, and much evidence has accumulated linking diabetes to exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Dioxin was the contaminant of the defoliant "Agent Orange", and has been found at many toxic waste disposal sites. Once diabetes is fully developed, it is often difficult to separate the effects of diabetes and hyperglycemia from the potential pathophysiologic events that led to this syndrome. In addition, risk factors for insulin resistance are very common, and it is difficult to dissect the overlapping influences of a toxic exposure in a complex human population. In this study, we sought to determine the relation between dioxin exposure and insulin resistance using a well-defined population. We measured insulin resistance in well-characterized veterans participating in the Air Force Health Study, an epidemiological investigation of veterans of Operation Ranch Hand.

## Methods and Materials

The Air Force Health Study is an ongoing 20-year prospective epidemiological study of veterans of Operation Ranch Hand, the Air Force unit responsible for aerial herbicide spray operations during the Vietnam conflict. Details of the study design and subject selection are described elsewhere[1]. 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 has been part of the study. In the full Air Force Health Study, Comparison veterans were matched to Ranch Hands with respect to age, race and military occupation.

All study subjects are male, and 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 health, mortality, and reproductive outcomes, and many of these results have been reported previously.

Beginning in 1987, blood from willing participants was collected and dioxin was measured in serum at the Centers for Disease Control and Prevention and expressed as parts per trillion (ppt) serum lipid. The dioxin measurements were done with high-resolution gas chromatography/high resolution mass spectrometry. The between assay coefficient of variation at three different concentrations of dioxin ranged from 9.4% to 15.5%.

To determine whether dioxin exposure was related to insulin resistance, we recruited veterans who completed the 1997 physical examination. In 1997, 2121 subjects were examined, of which 870 were Ranch Hand, and 1251 were Comparison veterans. From these, we limited our selection to those without diabetes or impaired glucose tolerance, based on a standard 75 g oral glucose tolerance test (fasting glucose <110, 2 hr glucose <140) at the 1997 physical. In addition, we selected from the Ranch Hand group those who had four previous measurements of serum lipid

dioxin, all of which were >10 ppt (n=71), and we selected from the Comparison group those whose dioxin level was <10 ppt (n=802).

For each of the 71 Ranch Hand veterans remaining we formed a matched set by matching two Comparison veterans from the 802 Comparison veterans. Matching was performed according to age (within 5 years), BMI (within 2 $kg/m^2$ at the 1997 physical examination), race (black or non-black), and a family history of diabetes (no vs yes for father, mother, sister, or brother) as reported on questionnaires administered in 1997. Seventy-one matched sets were identified, and 60 subjects from 30 matched pairs traveled to the University of Arkansas for Medical Sciences/ Central Arkansas Veterans HealthCare System, Little Rock, Arkansas, for insulin sensitivity testing. Prior to reporting for testing, subjects were interviewed by telephone, and fasting laboratory testing was performed. The interview elicited concurrent medical conditions, medications, and weight changes. Veterans who reported a weight gain or loss of more than 5% since the 1997 physical, a chronic or acute illness that may have affected insulin sensitivity (e.g. rheumatoid arthritis, recent myocardial infarction), taking medications likely to affect insulin sensitivity (e.g. corticosteroids), or abnormal liver or renal function, anemia, or electrolyte disturbances, were excluded.

Upon arrival, veterans were interviewed to review their medical history, skin fold thickness were measured at the triceps, subscapular, abdomen, waist, thigh, and calf, and body fat was measured by air plethysmography. Subjects spent a restful evening at the medical treatment facility, and were awakened at 0700 for insulin sensitivity testing, which was performed in the fasting state.

The measurement of *in vivo* insulin sensitivity was performed using the minimal model analysis of the frequently sampled intravenous glucose tolerance test (FSIVGTT)[2,3]. We used the classic tolbutamide-modified test that 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 minutes after the glucose injection, patients were given an injection of tolbutamide (125 $mg/m^2$) again followed by frequent blood sampling, according to a standard protocol. Together, 4 basal and 27 post-glucose blood samples were taken, the last one at 240 minutes. 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, Indiana). An insulin sensitivity index ($S_I$) was calculated using the MINMOD program, and expressed in units of minutes per μU/ml. Thirty pairs of subjects, 30 Ranch Hand veterans and a matched Comparison veteran traveled to Little Rock for these studies. Twenty-nine pairs were non-black and one pair was Black. Because one subject had an $S_I$ that was indeterminate secondary to poor insulin secretion, the data reported below are from 29 matched pairs.

We computed body mass index (BMI) as weight (kg) divided by the square of height (m). We used BMI computed from the 1997 physical examination as a matching variable. BMI at the end of each veteran's tour in Southeast Asia was computed from a review of medical records. BMI measured in 2000 for the 29 matched pairs was used as a covariate in adjusted analyses of $\log(S_I)$. We fitted three models with $\log(S_I)$ as the dependent variable in the 29 matched pairs. All three included adjustments for the paired feature of the study design. Model 1 was a paired t-test. In Models 2 and 3 the independent variable was $\log_2(\text{dioxin})-\log_2(10)$, which meant that the intercept was computed at dioxin=10 ppt. In Model 2, a single regression line was fit on the combined

cohort. In Model 3 separate regression lines were fit within each group and these lines had different intercepts while retaining a common slope. We conducted two-sided testing with a significance level of 5% throughout and used SAS® software (SAS Institute, Carey, NC) for all analyses and graphics.

**Results and Discussion**

The sample reduction is summarized in Table 1.

**Table 1.** Sample reduction for the matched pairs analysis of insulin sensitivity

| | Ranch Hand | | Comparison | |
|---|---|---|---|---|
| | Exclusions | Net | Exclusions | Net |
| Eligible[1] | | 567 | | 815 |
| Fewer than 4 dioxin values above 10 ppt[2]. | (496) | 71 | | |
| Dioxin above 10 ppt | | | (13) | 802 |
| Not matched with a Ranch Hand veteran | | | (662) | 140 |
| Not brought to Little Rock for testing | (41) | 30 | (110) | 30 |
| Matched Comparison's $S_I$ could not be determined | (1) | 29 | (1) | 29 |

1. After excluding recently deceased (n=33), those with missing dioxin measurements (n=20), diabetics (n=298), veterans with a recent myocardial infarction (n=10), and HIV (n=2) from those who attended the 1997 physical examination (n=2121).
2. parts per trillion.

Paired t-tests (Model 1) found no significant difference between cohorts with regard to the mean $S_I$ in original or log units (Table 2).

**Table 2.** Paired t-tests on the sensitivity index ($S_I$)

| Unit | Group | Mean (SEM) | Difference (SEM) | p-value |
|---|---|---|---|---|
| Original | Ranch Hand | 3.00 (0.41) | | |
| | Comparison | 3.26 (0.49) | -0.26 (0.58) | 0.65 |
| Log | Ranch Hand | 0.87 (0.13) | | |
| | Comparison | 0.90 (0.14) | -0.035 (0.17) | 0.84 |

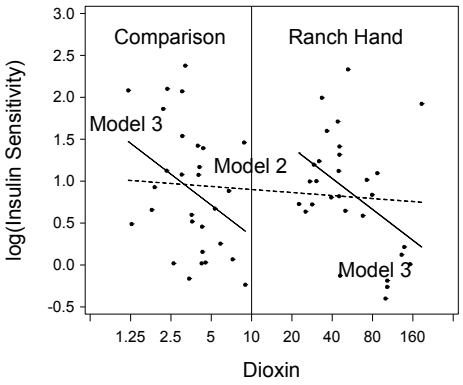
For Model 2, where we considered $\log(S_I)$ and $\log_2$(dioxin) in the combined cohort, there was no significant linear relation (r=-0.17, p=0.38, Figure 1). For Model 3, where we considered a linear regression of $\log(S_I)$ on $\log_2$(dioxin) within each group separately, we found a significant linear relation common to both groups (r=-0.46, p=0.011, Figure 1), but with different intercepts (p=0.016). We reanalyzed with adjustment for age and BMI, and the change in BMI from the end of the Southeast Asia tour to 2000 but the changes were negligible. Because the within-group slopes were similar (Ranch Hand: slope=-0.376, Comparison: slope=-0.363) and not significantly different (p=0.97), we considered a common slope in Model 3.

**Table 3.** Regression coefficients for $\log(S_I)$ vs $\log_2$(dioxin).

| | Model 2 ($R^2$=0.03, p=0.38) Regression on the combined cohort[1] | | Model 3 ($R^2$=0.22, p=0.04) Regressions within each cohort[2] | |
|---|---|---|---|---|
| Parameter | Estimate (SE) | p-value[3] | Estimate (SE) | p-value[3] |
| Intercept(s) | 0.900 (0.086) | <0.001 | C:[4] 0.347 (0.230) | 0.14 |
| | | | R:[4] 1.768 (0.348) | <0.001 |
| Slope | -0.036 (0.041) | 0.38 | -0.368 (0.135) | 0.011 |
| Correlation | -0.17 | 0.38 | -0.46 | 0.011 |

1. A single regression line with the intercept measured at dioxin=10 ppt.
2. Separate regression lines in each cohort having different intercepts (designated C: and R:) but a common slope.
3. P-value for test that regression coefficient equals 0.
4. The C and R intercepts were significantly different (p=0.016).

**Figure 1.** Regression lines relating insulin sensitivity and dioxin in 29 matched pairs.




These studies were intended to determine whether Vietnam veterans who were matched according to age, BMI, and family history of diabetes, and who differed primarily on serum levels of dioxin, demonstrated differential degrees of insulin resistance. As shown in Table 2, we found no significant difference in the mean $\log(S_I)$ between groups. It should be noted that the original exposure to dioxin was more than 30 years ago, and we excluded veterans with diabetes. Hence we may have excluded those who were most susceptible to potentially toxic effects of dioxin. Notwithstanding the absence of a significant difference in mean $\log(S_I)$ between groups, we found a significant linear relation between $\log(S_I)$ and $\log_2$(dioxin) within each group (Model 3). These effects could not be explained by other established risk factors for diabetes (such as age and BMI). Indeed the two groups were similar with regard to age and there were not large variations in BMI. A number of explanations for these patterns are possible. Dioxin level, for example, may be a marker for some other phenomenon that is associated with insulin resistance. Dioxin elimination may reflect physiological parameters that also affect $S_I$. No relation between the dioxin elimination rate and the risk of diabetes in Ranch Hand veterans[4] has been found, however.

**References**

1. Wolfe W.H., Michalek J.E., Miner J.C., Rahe A., Silva J., Thomas W.F., Grubbs W.D., Lustik M.B., Karrison T.G., Roegner R.H. and Williams D.E. (1990) JAMA 264, 1824-1831
2. Bergman R.N., Hope I.D., Yang Y.J., Wantanabe R.M., Meador M.A., Young J.H. and Ader M. (1989) Diabetes Metab Rev 5,411-429
3. Bergman R.N., Beard J.C., and Chen M. (1986) The minimal modeling method: assessment of insulin sensitivity and B-cell function in vivo. In Methods in diabetes research, Volume II. J. Larner, W.L. Clarke, and S.L. Pohl, editors. John Wiley and Sons, New York 15-34
4. Michalek J.E., Ketchum N.S. and Tripathi R.C. (2003) J Toxicol Environ Health A 66(3), 211-21