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Insulin Sensitivity following Agent Orange Exposure in Vietnam Veterans with High Blood Levels of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

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Our objective was to determine whether insulin sensitivity was related to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Vietnam veterans exposed to Agent Orange. Air Force veterans of Operation Ranch Hand, the unit responsible for spraying Agent Orange and other herbicides in Vietnam from 1962 to 1971, and comparison veterans who did not spray herbicides were included. We measured insulin sensitivity (S_I) using a frequently sampled iv glucose tolerance test in a matched study of 29 matched pairs of veterans and a quantitative insulin sensitivity check index (QUICKI) based on fasting glucose and insulin in 71 matched pairs. No group differences were found with regard to the mean values of S_I, QUICKI, TNFα, adiponectin, and two measures of insulin secretion. However, S_I and QUICKI decreased significantly with regard to TCDD ($P = 0.01$ and 0.02). A corresponding pattern (although not significant) was found for blood levels of TNFα and adiponectin. These data suggest that high blood TCDD levels may promote an insulin-resistant state, but the magnitude of this effect appeared to be small, such that an 18-fold increase in blood TCDD due to increased exposure resulted in only a 10% change in S_I in the 29 matched pairs. (*J Clin Endocrinol Metab* 89: 4665–4672, 2004)

BETWEEN 1965 AND 1971, the U.S. Air Force sprayed 17.6 million gallons Agent Orange and other herbicides 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 ppm. 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 fixed-wing aircraft in Vietnam.

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). A study of diabetes and TCDD in 279 chemical workers involved in the production of trichlorophenol, 2,3,5-T and hexachlorophene conducted by the National Institute for Occupational Safety and Health found a nonsignificantly increased risk of diabetes in exposed workers, but no dose-response trend with serum TCDD (4). However, of the 10 workers with the highest current TCDD concentrations, six had diabetes mellitus. In a study from Seveso, Italy, in which 45,000 people had varying levels of exposure to TCDD, there were significant increases in mortality from coronary artery disease and diabetes (5). Several studies (6, 7) demonstrated a relationship between blood TCDD levels and hyperinsulinemia. The data suggest that nondiabetic 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. The Institute of Medicine concluded that there is limited/suggestive evidence of an association between exposure to herbicides used in Vietnam or the TCDD contaminant and diabetes (7). Based on the Institute of Medicine opinion, the Department of Veterans Affairs decided that type 2 diabetes is a service-connected condition in Vietnam veterans.

Although the precise pathogenesis of type 2 diabetes is unknown, the initial pathophysiologic event is usually insulin resistance, a condition found in essentially all people who eventually become diabetic and that develops early in the course of the disease (8, 9). Insulin resistance is widely prevalent in developed countries due to the presence of common genetic susceptibility genes, coupled with obesity and a sedentary lifestyle. Although insulin resistance is widespread, not all insulin-resistant individuals develop diabetes. In susceptible individuals, insulin resistance leads to or is accompanied by insulin secretory failure, which eventually leads to impaired glucose tolerance, and finally fasting hyperglycemia and type 2 diabetes mellitus (10). Recent studies (11, 12) demonstrated an association between the expression of various inflammatory cytokines, such as TNFα, IL-6, and adiponectin, and the insulin resistance syndrome, suggesting

Abbreviations: AIR_G, Acute insulin response to glucose; BMI, body mass index; FSIVGTT, frequently sampled iv glucose tolerance test; POP, persistent organic pollutant; ppt, parts per trillion; QUICKI, quantitative insulin sensitivity check index; S_I, insulin sensitivity index; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ, toxic equivalent.

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that a proinflammatory state is present in individuals at high risk for diabetes.

One mechanism by which TCDD produces biological effects is through up-regulating TNFα expression in several different cell types (13, 14), with the toxic effects of TCDD treatment being a direct result of increased TNFα expression. For example, administration of anti-TNFα antibody resulted in less TCDD-induced oxidative stress in hepatic nuclei (15), and anti-TNFα antibodies have also been found to reduce TCDD-mediated mortality in mice (16). These findings suggest that a connection between TCDD and TNFα expression may be the key to understanding TCDD-mediated insulin resistance. Other possibilities for TCDD-mediated diabetes involve an inhibition of peroxisomal proliferator-activated receptor-γ through the Ah receptor (17).

Previous studies have shown that adipose TNFα is related to insulin resistance. When compared with lean littermates, rodents with genetic obesity and insulin resistance expressed 5- to 10-fold more TNFα mRNA and 2 times more TNFα protein in their adipose tissue (18). In an attempt to reverse the insulin resistance in these animals, a soluble TNF-binding protein was infused into *fa/fa* rats. This resulted in a 2- to 3-fold increase in insulin-stimulated glucose uptake (18) along with decreased plasma insulin and nonesterified fatty acid levels and increased autophosphorylation of the insulin receptor (tyrosine kinase) and insulin receptor substrate 1 in both adipose tissue and muscle (19). In addition, TNFα knockout mice and mice that lack the TNFα receptor fail to become insulin resistant when placed on a high-fat diet (20), suggesting that adipose TNFα causes insulin resistance when combined with a high-fat diet. To demonstrate the relevance of rodent studies to humans, studies examined TNFα expression in humans. Obese subjects had elevated levels of adipose TNFα mRNA and protein, which decreased with weight loss (21, 22). Together, these studies suggest that TNFα overproduction by adipose tissue was involved in the pathogenesis of the insulin resistance of obesity, and TCDD stimulates TNFα expression, which may then promote insulin resistance.

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 separate them from the overlapping influences of a toxic exposure in a complex human population. Hence, in this study, we wished to study TCDD exposure and insulin resistance using a well-defined population. We measured insulin sensitivity using two different methods in well-characterized Vietnam veterans who have been participating in the Air Force Health Study, a prospective epidemiological study of veterans of Operation Ranch Hand.

Subjects and Methods

The Air Force Health Study is an ongoing prospective epidemiological study that seeks to determine whether 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. Details of the study design and subject selection are described elsewhere (23). 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 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 (3, 24–27), mortality experience (28–29), and reproductive outcomes (30–33). The current study was conceived because previous studies suggested a relationship between TCDD levels and diabetes (3).

Blood from willing participants was collected, and TCDD was measured in serum at the Centers for Disease Control and Prevention and expressed as parts per trillion (ppt) serum lipid (34). The serum TCDD measurements were done with high-resolution gas chromatography/high-resolution mass spectrometry (35, 36). 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 yr (37).

We studied insulin sensitivity in two sets of selected Ranch Hand and comparison veterans who participated in the 1997 and 2002 physical examinations. The 1997 study was a matched-pair study of 30 one-to-one matched pairs, selected from veterans who attended the 1997 physical examination. The 2002 study was a matched study of 71 one-to-one matched pairs, selected from veterans who attended the 2002 physical examination. In both studies, one Ranch Hand was matched to one comparison, and insulin sensitivity was measured in each veteran. In the 1997 study, the frequently sampled iv glucose tolerance test was used, and in the 2002 study, a quantitative insulin sensitivity check index (QUICKI) (38) was used, as described below. In both studies, a body mass index (BMI) was computed as weight (kilograms) divided by the square of height (meters).

The 1997 study

During the 1997 physical examination, a 75-g oral glucose tolerance test was performed. As shown in Table 1, we limited our selection of subjects to those without diabetes or impaired glucose tolerance, based on a standard 75-g oral glucose tolerance test (fasting glucose < 110 mg/dl, 2-h postprandial glucose < 140 mg/dl). We restricted this study to nondiabetic veterans because an earlier study found mean postprandial insulin increased in nondiabetic Ranch Hand veterans with high TCDD exposure (24). In this study design, we intended 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 yr), BMI (within 2 kg/m^2), race (black, nonblack), and a family history of diabetes in first-order relatives (yes, no) as reported on questionnaires administered at the 1997 physical examination. As described in Table 1, the cohort of 870 Ranch Hand and 1251 comparison veterans was reduced to 29 matched pairs through exclusions due to death; missing TCDD results; health conditions previously mentioned; and, in Ranch Hands, fewer than four TCDD results greater than 10 ppt, comparisons that could not be matched to a Ranch Hand, and individuals not scheduled due to noncompliance of the opposite member of the pair or the end of the study accrual phase.

Thus, the Ranch Hand subjects had had consistently high TCDD levels since at least 1982, and the comparison group had low TCDD levels.

Before 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: 1) a weight gain or loss of more than 5% since the 1997 physical examination, 2) 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), 3) taking medications likely to affect insulin sensitivity (such as corticosteroids), and 4) the occurrence of liver abnormalities, renal dysfunction, anemia, or electrolyte disturbances.

Sixty veterans (comprising the 30 matched pairs) traveled to the

TABLE 1. Sample reduction

	1997 Matched study		2002 Matched study	
	Ranch Hand	Comparison	Ranch Hand	Comparison
Attended the 1997 physical examination	870	1251	870	1251
Died	11	24	11	24
No TCDD result	5	15	5	15
Diabetic prior to August 1999	124	174	124	174
Impaired glucose tolerance[a]	156	218	156	218
Recent myocardial infarction[b]	6	4	6	4
HIV positive	1	1	1	1
Ranch Hand with fewer than 4 TCCD results > 10 ppt or Comparison with at least one TCDD result > 10 ppt	496	13		
Did not attend the 2002 examination			62	74
Diabetic after 1999			3	12
BMI at the end of Southeast Asia tour not available			5	6
Comparison could not be matched to a Ranch Hand		662		
Not tested[c]	41	110		
Comparison SI could not be determined and matched Ranch Hand	1	1		
Age ≥ 70 yr at the 2002 physical examination			83	112
Ranch Hand with TCDD ≤ 10 ppt or Comparison with TCDD > 10 ppt			206	9
Fasting insulin below the detection limit			0	1
Could not be matched within specified limits			137	530
Net	29	29	71	71

[a] Fasting glucose ≥ 110 mg/dl or 2-h postprandial glucose ≥ 140 mg/dl at the 1997 physical examination.
[b] Diagnosed after January 1998.
[c] Either one or both members of a matched pair could not be scheduled or not scheduled because the prescribed number of 30 matched pairs had already been tested.

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 h 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 iv glucose tolerance test (FSIVGTT) (39, 40). We used the classic tolbutamide-modified test, which has been validated against the euglycemic clamp in humans (41, 42). In brief, catheters were placed for glucose injection, and 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, four basal and 27 post-glucose blood samples were taken, the last one at 240 min. Glucose was measured using a glucose oxidase method in a glucose analyzer, and insulin was measured using a RIA. 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 (41, 42) and expressed in units of minutes^{-1}/(microunits per milliliter). The acute insulin response to glucose (AIR_G) was also determined as the area under the insulin curve during the first 2–10 min after the glucose injection (milligrams per minute per deciliter). A disposition index was computed as the product of AIR_G and S_I. The disposition index is a measure of β-cell compensation for changes in insulin sensitivity (43). 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 (picograms per milliliter) and adiponectin (micrograms per milliliter) were measured in each member of each pair. The measurement of adiponectin protein employed a RIA (Linco Research, St. Charles, MO). This assay demonstrates a 4.3% intraassay variation, and a 7.1% interassay variation. TNFα was measured using ELISA (R&D Systems, Minneapolis, MN).

The 2002 study

In the 2002 study, we measured fasting insulin and glucose in veterans who volunteered for the 2002 physical exam. Using these data, we calculated QUICKI (37), defined as 1/[log(fasting glucose) + log(fasting insulin)]. Fasting glucose (milligram per deciliter) was measured using the glucose oxidase method (Dade Behring, Newark, DE), and fasting insulin (milligram per deciliter) was measured by RIA (Diagnostic Products Corp., Flanders, NJ). As described in Table 1, exclusion of veterans with newly diagnosed diabetes, age older than 70 yr (44), or missing BMI from the tour of duty in Southeast Asia from those who attended the 2002 examination provided a cohort of 809 veterans for consideration in a matched analysis of insulin resistance using the QUICKI. These were matched one-to-one on race (black, nonblack), family history of diabetes in first-order relatives (yes, no) as reported in 2002, age (to within 5 yr), and BMI at the 2002 physical examination (to within 2 kg/m^2), resulting in 71 matched pairs, with one Ranch Hand matched to one comparison veteran.

Statistical methods

In the 1997 study, 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. In the 2002 study, we analyzed QUICKI in original units.

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

Based on the 1997 physical examination, 29 matched pairs of subjects successfully completed insulin sensitivity testing using the FSIVGTT with minimal model analysis. Table 2 summarizes the demographic and metabolic characteristics of the 29 matched pairs. There were no significant differences in mean age; BMI; percentage with a family history of diabetes; or mean hemoglobin A_1C, triglycerides, cholesterol, high-density lipoprotein cholesterol, fasting glucose, or fasting insulin. Even though the basis of the matching included

TABLE 2. Characteristics of 29 matched pairs of Air Force veterans who attended the 1997 Air Force Health Study physical examination

Characteristic	Ranch Hand (n = 29)	Comparison (n = 29)
Mean age (yr) at insulin sensitivity testing (SD)	56.6 (6.4)	56.8 (6.1)
BMI at insulin sensitivity testing, mean (SD)	30.9 (3.5)	31.2 (3.2)
African-American (%)	1 (3.3)	1 (3.3)
Reported family history of diabetes (%)[a]	27.6	27.6
Military occupation category (%)		
Officer	3.5[d]	24.1
Enlisted flyer	17.2	13.8
Enlisted ground crew	79.3	62.1
Mean HbgA1c (SD)[a]	6.1 (0.6)	6.2 (0.5)
Median triglycerides (range) (mg/dl)[a]	125 (47–445)	113 (49–217)
Mean cholesterol (SD) (mg/dl)[a]	216 (32)	220 (35)
Mean HDL cholesterol (SD) (mg/dl)[a]	45.3 (7.5)	45.1 (11.2)
Median TCDD (range) (parts per trillion)	45.3[d] (22.6–186)	3.6 (1.2–9.0)
Mean fasting insulin (SD) (μU/ml)[b]	13.3 (9.6)	11.3 (5.0)
Mean fasting glucose (SD) (mg/dl)[b]	94.1 (9.8)	94.9 (10.4)
Median insulin sensitivity (S_I) (range) (min^{-1}/μU/ml)	2.3 (0.7–10.3)	2.4 (0.8–10.8)
Median acute insulin response to glucose (AIR_G) (range) (mg·min/dl)	511 (85–2232)	508 (33–1383)
Median disposition index ($S_I \times AIR_G$) (range)	1356 (70–4262)	1107 (101–4066)
Median TNFα (range) (pg/ml)[c]	2.77 (1.36–8.93)	2.62 (1.49–6.01)
Mean adiponectin (SD) (μg/ml)[c]	8.55 (4.35)	9.09 (3.44)

[a] At the 1997 physical examination.
[b] At the 2002 physical examination.
[c] In 20 pairs with sufficient serum collected from both members to make the measurement.
[d] $P < 0.05$ *vs.* comparison group.

TABLE 3. Characteristics of 71 matched pairs of Air Force veterans who attended the 2002 Air Force Health Study physical examination

Characteristic	Ranch Hand (n = 71)	Comparison (n = 71)
Mean age (yr) (SD)[a]	57.6 (4.1)	58.4 (4.6)
Black (%)	7.0	7.0
Mean BMI (SD)[a]	28.1 (3.8)	27.4 (3.9)
Mean BMI at end of SEA tour (SD)	23.5 (2.2)	23.8 (2.5)
Reported family history of diabetes in 2002 (%)	39.4	39.4
Military occupation category (%)		
Officer	16.9[b]	32.4
Enlisted flyer	14.1	7.0
Enlisted ground crew	69.0	60.6
Mean TCDD (range) (parts per trillion)	25.4[b] (10.3 to 58)	3.7 (0.5 to 8.7)
Median fasting insulin (range) (μU/ml)[a]	11 (4 to 41)	9 (3 to 63)
Mean fasting glucose (SD) (mg/dl)[a]	96.1 (9.4)	95.5 (10.9)
Median triglycerides (range) (mg/dl)[a]	118 (48 to 530)	121 (51 to 1215)
Mean QUICKI (SD)[c]	0.336 (0.027)	0.342 (0.029)

[a] At the 2002 physical examination.
[b] $P < 0.05$ *vs.* comparison group.
[c] QUICKI = 1/[log(fasting glucose) + log(fasting insulin)].

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.

At the time of the 2002 physical examination, more subjects were available for study, and 71 matched pairs of Ranch Hand and comparison Air Force veterans were studied. Table 3 summarizes the demographic and metabolic characteristics of these 71 matched pairs. The Ranch Hand median TCDD was greater than the comparison median, by design, and a smaller percentage of Ranch Hand veterans were officers during the War than comparison veterans. The groups were similar on the other variables listed in Table 3.

In the 29-pair study based on the 1997 physical, there were no significant differences in mean S_I, AIR_G, or disposition index between Ranch Hand and comparison veterans, nor were there differences in blood adiponectin or TNFα (Table 4). Similarly, in the 71-pair study based on the 2002 physical examination, there was no significant difference in the mean insulin sensitivity as represented by QUICKI (Table 4).

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, and the gradual 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 of both matched-pair cohorts. Within-pair differences in the dependent variables were regressed on within-pair differences (Ranch Hand minus com-

TABLE 4. Group mean contrasts and regression model results

A) 1997 matched pair study
a) Mean within-pair differences[a]

Dependent variable	Mean difference (SE)	95% CI	*P* value
$\log(S_I)$	−0.035 (0.170)	−0.384 to 0.314	0.84
$\log(AIR_G)$	0.069 (0.191)	−0.322 to 0.459	0.72
$\log(\text{disposition index}) = \log(S_I \times AIR_G)$	0.034 (0.231)	−0.440 to 0.508	0.88
$\log(TNF\alpha)$	0.011 (0.132)	−0.265 to 0.286	0.94
Adiponectin	−0.543 (1.151)	−2.952 to 1.866	0.64

b) Linear regressions on within-pair differences[a,b]

Dependent variable	Intercept (SE)	Slope (SE)	Slope 95% CI	*P* value
$\log(S_I)$	1.42[c] (0.56)	−0.368 (0.135)	−0.645 to −0.091	0.01
$\log(AIR_G)$	−0.62 (0.69)	0.174 (0.167)	−0.169 to 0.518	0.31
$\log(\text{disposition index}) = \log(S_I \times AIR_G)$	0.80 (0.84)	−0.194 (0.204)	−0.613 to 0.224	0.35
$\log(TNF\alpha)$	−0.65 (0.40)	0.172 (0.100)	−0.038 to 0.383	0.10
Adiponectin	5.35 (3.50)	−1.541 (0.871)	−3.371 to 0.289	0.09

B) 2002 matched pair study
a) Mean within-pair difference[a]

Dependent variable	Mean difference (SE)[a]	95% CI	*P* value
QUICKI	−0.0056 (0.0041)	−0.014 to 0.003	0.18

b) Linear regression on within-pair differences[a,b]

Dependent variable	Intercept (SE)	Slope (SE)	Slope 95% CI	*P* value
QUICKI	0.015 (0.010)	−0.00639 (0.00270)	−0.012 to −0.001	0.02

[a] For both the group mean contrast and the regression model, within-pair differences were computed as the Ranch Hand value minus the comparison value.
[b] For the regression model, the independent variable was the Ranch Hand TCDD minus the comparison TCDD value in log units.
[c] Significantly different from zero ($P = 0.02$).

parison) in TCDD levels in log (base 2) units, and these analyses are shown in Table 4. Slopes relating within-pair differences of dependent variables to within-pair differences on TCDD were negative and reached significance for S_I ($P = 0.01$) and QUICKI ($P = 0.02$). Stated differently, pairs with the greatest difference in TCDD levels demonstrated the largest decrease in S_I or QUICKI and hence the largest amount of insulin resistance. The regression lines for S_I and QUICKI are shown in Figs. 1 and 2. Using this analysis, we attempted to examine the magnitude of the effect of blood TCDD level on insulin resistance. Based on the data in Fig. 1, 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 our 1997 matched pair study.

In addition, using the same analysis, there was a 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.

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

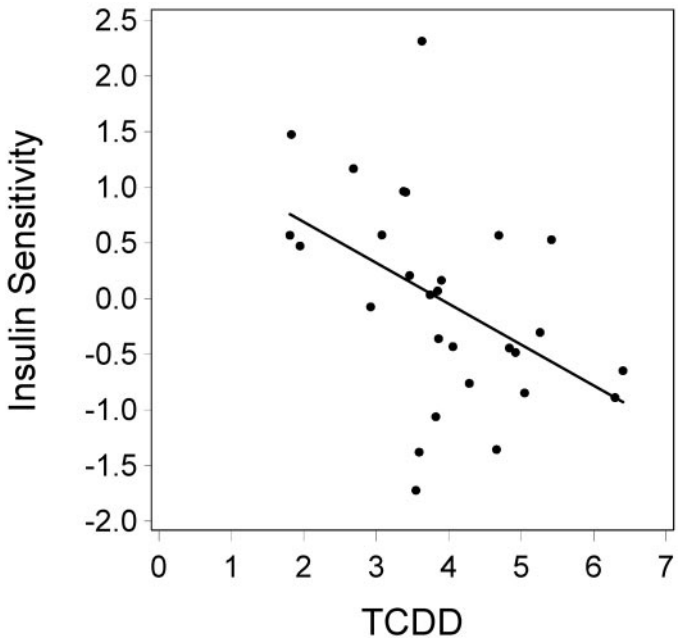

FIG. 1. Within-pair differences in S_I in log units *vs.* within-pair differences in TCDD in log base 2 units in the 1997 study of 29 matched pairs. Within-pair differences were computed as the Ranch Hand value minus the comparison value.

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 (3, 5, 6). There are a number of possible mechanisms for TCDD-mediated

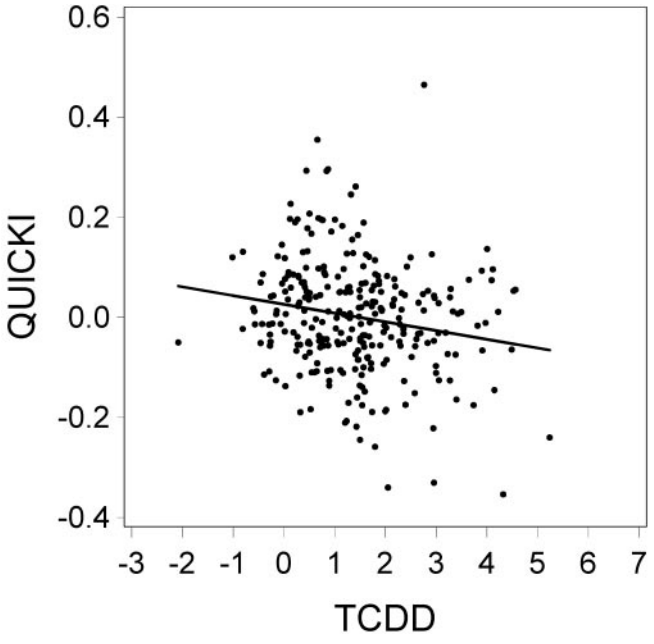

FIG. 2. Within-pair differences in QUICKI in original units *vs.* within-pair differences in TCDD in log base 2 units in the 2002 study of 71 matched pairs. Within-pair differences were computed as the Ranch Hand value minus the comparison value.

insulin resistance. A number of studies *in vitro* have demonstrated an increase in cellular expression of TNFα after exposure to TCDD (16, 45). 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 unusual because of the extensive characterization of the participants and the measurement of blood TCDD levels in both the index and control groups. These studies were 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 S_I and related parameters. We found no significant mean differences in either measure of 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 S_I or a subtle effect that cannot be detected using this method of analysis. We also found no significant mean differences with regard to the disposition index, which measures β-cell compensation for changes in insulin resistance. Because of the subtle changes in S_I, we did not expect to see significant differences in β-cell compensation. To determine whether there was a subtle effect of TCDD on S_I, we examined within-pair differences on S_I and QUICKI and found that S_I decreased significantly with regard to within-pair differences on TCDD. These changes in S_I were accompanied by a trend toward changes in the plasma level of cytokines TNFα and adiponectin that would be consistent with a TCDD-mediated worsening or S_I.

It is possible that there is no real association between TCDD, and any of these variables and the within-pair differences might be an artifact of chance or lack of adjustment for some other unmeasured variable. Diabetes and insulin resistance are complex conditions that are affected by numerous genetic and environmental conditions. Alternatively, TCDD may actually play an active but relatively small role in contrast to other established risk factors for insulin resistance. For example, our regression model predicted only a 10% decrease in the S_I for a 18-fold difference in TCDD levels between a Ranch Hand and his matched comparison in our 1997 matched pair study. However, this 10% reduction in S_I would be present only in the relatively small fraction of the population with very high TCDD levels. If we assume a mean blood TCDD level of 3 ppt among Americans with incidental exposure, then it would take a blood level of 54 ppt to obtain a 10% decrease in the S_I. Among 1524 comparison veterans in the Air Force Health Study, one (or 0.066% of the comparison cohort) had a TCDD level greater 54; his TCDD level was actually 54.8 ppt, suggesting that our dose-response model would apply to only a fraction of 1% of the American male population. For the majority of the population with low serum TCDD levels, our data would predict a negligible effect on S_I. In subjects with very high levels of TCDD, a 10% reduction in S_I could be related to the risk of diabetes; however, other factors, such as obesity, would likely cause a greater change in the S_I. It is plausible that the TCDD elimination rate may be affected by physiological parameters, such as BMI, that also influence S_I. However, in previous studies, no relation between the TCDD elimination rate and the risk of diabetes in Ranch Hand veterans (46) has been found, and neither of the measures of S_I studied here was related to the TCDD elimination rate (data not shown).

Other exposure metrics could be considered to characterize TCDD exposure. We used TCDD measured in 1987 and subsequently. The peak concentration at the time of service in Vietnam and the area under the curve may also be of interest. We estimated the peak concentration in the 29 Ranch Hand subjects using the 1987 TCDD and using a first-order model with a half-life of 7.6 yr. With this measure of TCDD exposure, none of the effects studied in this paper, including means and slopes, was statistically significant. Analyses based on the area under the curve also showed no significant results. We would tend to discount these analyses, however, because TCDD measurements made in 1987 and subsequently may be more relevant to the clinical chemistry measurements studied here than estimates of the peak TCDD dose that occurred up to 40 yr earlier.

The Air Force Health Study was launched in 1980, and the first TCDD measurements were made in 1987. Of the many persistent organic pollutants (POPs) known today, we measured only TCDD because the purpose of the study was to address health and exposure to Agent Orange and its TCDD contaminant. For the past 20 yr, toxicologists have studied other POPs including dioxin congeners, furans, and polychlorinated biphenyls and have summarized these in a calculated measure known as the toxic equivalent (TEQ) (47). Because other POPs are known to be endocrine disrupters and may also influence the S_I, a hypothetical relation between the S_I and the TEQ is of interest. We were unable to

address S_I and the TEQ in this study because we measured only TCDD. For example, the TEQ is not a simple multiple of TCDD. In victims of the Seveso accident (48), the ratio of TCDD to TEQ ranged from 7 to 14% in pooled serum from female residents aged 20–40 yr of zone non-ABR, and the variation between individuals in this ratio would probably be greater. In our study, we do not know the amount or kind of other chemical exposures these veterans received; therefore, we cannot estimate the contribution of the dioxin congeners, furans, and polychlorinated biphenyls to the TEQ for an individual.

Another possible explanation for the lack of a significant mean difference on S_I and QUICKI could be related to the study design, which excluded patients with diabetes or impaired glucose tolerance, and the long period of time since TCDD exposure. If, for instance, TCDD exposure accelerated the development of insulin resistance and diabetes in susceptible subjects, then susceptible subjects with high TCDD exposure may have already developed diabetes or impaired glucose tolerance and hence were not included in this study, leaving behind the Ranch Hand subjects who are generally less susceptible to diabetes based on the complex genetic and environmental factors that contribute to this disease.

In conclusion, we measured SI using a FSIVGTT (S_I) in a study of 29 matched pairs of Ranch Hand and comparison veterans who attended the 1997 examination and the QUICKI in 71 matched pairs who attended a physical examination in 2002. We found no significant difference between comparison and Ranch Hand veterans in mean S_I or QUICKI. However, both S_I and QUICKI decreased significantly with regard to TCDD in the adverse direction. The same pattern of an adverse trend with TCDD was found for TNFα 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.

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