SERUM DIOXIN LEVEL IN RELATION TO DIABETES MELLITUS AND GLUCOSE INTOLERANCE AMONG AIR FORCE VETERANS WITH BACKGROUND LEVELS OF EXPOSURE

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Introduction

One of the most detailed investigations of the health effects of dioxin is the Air Force Health Study (AFHS). The AFHS is a systematic study of male Air Force veterans who participated in Operation Ranch Hand, and of a comparable group of male Air Force veterans who served in southeast Asia during the same period. Operation Ranch Hand involved potential exposure to the herbicide, Agent Orange, which was contaminated with dioxin. As part of the AFHS, serum dioxin level was measured in over 2000 men. The 1992 examination of these veterans included assessment of whether they had diabetes, and a measurement of serum glucose while fasting, and two hours after an oral bolus of glucose (glucose tolerance test).

In the AFHS, an increased risk of diabetes mellitus and impaired glucose tolerance was found among veterans of Operation Ranch Hand who had high serum levels of dioxin[1]. Here we report that the association between serum dioxin level and diabetes and impaired insulin-glucose metabolism was apparent among those in the AFHS comparison group with background levels of exposure to dioxin[2].

Materials and Methods

In the AFHS, Air Force veterans who participated in Operation Ranch Hand or who were deemed comparable and served in southeast Asia during the time of the Operation (1962-1971) have been subject to careful scrutiny with respect to level of exposure to dioxin and health status. The veterans in the comparison group were matched to veterans of Operation Ranch Hand by age, race, and military occupation. All veterans of Operation Ranch Hand were male, thus all veterans in the AFHS were male. Physical examinations and interviews have been conducted periodically since 1982. The 1987 examination included measurement of serum dioxin level. The 1992 examination included an assessment of carbohydrate metabolism, and measurement of serum dioxin for veterans whose dioxin level had either not been previously measured or was previously measured but not quantifiable (n=274). Additional veterans had dioxin measured in 1997 for the same reason (n=21). For those veterans whose dioxin was measured after 1987, the subsequent measure was substituted for the missing 1987 value. Diagnosis of diabetes was based either on self-report verified by medical records or a postprandial glucose of $\geq$ 200 mg/dl.

Of the 1667 comparison group veterans invited to participate in the first AFHS examination in 1982, 73% complied. Of the 1731 comparison veterans invited to participate in the 1987 examination, 75% complied. Of the 1762 comparison veterans invited to participate in the 1992 examination, 73% (n=1281) complied. Of the 1,281 comparison veterans examined in 1992, we excluded those with missing data for dioxin (n=11), waist size (n =15), postprandial glucose (n=1),

or triglycerides at the time the dioxin was measured (n=33). For all other items of interest, such as body mass index, the data were complete. Finally, we excluded comparison veterans whose serum dioxin level was > 10 ng/kg lipid (n=24), because such levels suggest that their exposure was above the background range. Thus, 1197 comparison veterans with complete data for the covariates of interest (Table 1) were included in the analysis.

The multivariate-adjusted results presented were adjusted for age, body mass index in 1992, body mass index at the time of the blood draw for dioxin, and waist in 1992 as continuous variables. In addition, all results were adjusted for race (white, black), military occupation (officer, enlisted flyer, enlisted ground crew), and family history of diabetes (no, yes) as categorical variables. The effect of adjusting associations for serum triglycerides at the time of the dioxin blood draw was considered in detail for several reasons, including the possibility that the composition of serum lipid might affect the dioxin level expressed on a per lipid basis. We scaled the odds ratios and regression coefficients presented in the tables so that they represented the increment in the outcome associated with an increase of the median level of serum dioxin in the study population, to reflect the effect as compared with what would be observed in the general population in the absence of dioxin contamination.

Results and Discussion

Most of the 1197 veterans included in the analysis were middle-aged white males, more than half were overweight (body mass index [BMI] $\geq$ 25 kg/m^2), and more than a quarter were obese (BMI $\geq$ 30 kg/m^2) (Table 1). About 14% of the veterans had diabetes type II, and 24% had a family history of diabetes.

Table 1. Characteristics of 1,197 background-exposed subjects who participated in the 1992 examination and who had complete data.

			Quartiles	
Characteristics	Percent in category	Median	1	3
Age		53	47	59
Black (%)	5.3			
BMI (kg/m^2)		28	26	31
Waist (cm)		98	92	106
Diabetic (%)	14.1			
Family history of Diabetes (%)	23.7			
Dioxin (ng/kg) in 1987		4.0	2.8	5.2
BMI (kg/m^2) in 1987		28	25	30
Nondiabetic subjects (n=1,028)				
Fasting serum glucose(mg/dl)		98	94	103
Postprandial Serum glucose (mg/dl)		103	88	123
Insulin (μIU/mL)		68	39	123

The multivariate-adjusted odds ratio for diabetes increased with level of serum dioxin (Table 2). After adjustment for serum triglycerides at the time blood was drawn for dioxin measurement the

association was attenuated somewhat. For the previously diagnosed diabetics (n=93) the odds ratio was 1.6, and for those diagnosed on the basis of the glucose tolerance test in 1992 (n=76), the odds ratio was 1.4.

Table 2. Multivariate adjusted odds ratio (OR) for diabetes per 4.0 ng/kg lipid increase in serum dioxin level, with and without adjustment for serum triglycerides.

	Multivariate Adjusted	
	OR	95% CI
Not adjusted for triglycerides	1.55	1.09, 2.20
Adjusted for triglycerides	1.37	0.96, 1.97

The multivariate-adjusted regression coefficients for glucose and insulin showed a positive relation between level and serum dioxin (Table 3), though for glucose the lower bound of the confidence intervals were near zero. After adjustment for serum triglycerides at the time blood was drawn for dioxin measurement, the associations were attenuated.

Table 3. Multivariate adjusted change in level of serum glucose or insulin (and 95% CI) among nondiabetics (n=1,102) per 4.0 ng/kg lipid increase in serum dioxin, with and without adjustment for triglycerides.

		Multivariate Adjusted	
		OR	95% CI
Glucose (mg/dl)	Fasting		
	Not adjusted for triglycerides	0.95	-0.17, 2.07
	Adjusted for triglycerides	0.74	-0.39, 1.88
	Postprandial		
	Not adjusted for triglycerides	3.0	-0.33, 6.40
	Adjusted for triglycerides	2.0	-1.36, 5.41
Insulin* (μIU/mL)	Postprandial		
	Not adjusted for triglycerides	0.11	0.016, 0.208
	Adjusted for triglycerides	0.065	-0.030, 0.161

* The natural logarithm of insulin was modeled.

Exposure to background levels of the ubiquitous environmental contaminant dioxin was associated with increased prevalence of diabetes and impaired insulin-glucose metabolism in these data. If this were a causal relation, a possible mechanism might be that dioxin exposure impairs metabolism, causing hypertriglyceridemia and increased insulin-glucose levels. If so, adjustment of the dioxin-diabetes association for triglycerides is not appropriate.

Several plausible noncausal mechanisms could also account for the observed association between dioxin and impaired insulin-glucose metabolism. Imagine, for example, that impaired metabolism causes not only an increase in serum lipids but a change in lipid composition, such that for a given concentration of total serum lipid, a greater proportion was comprised of triglycerides, increasing

the solubility of dioxin. If dioxin exposure causes hypertriglyceridemia and increased insulin-glucose levels, adjustment of the dioxin-diabetes association for triglycerides is not appropriate.

Even if triglycerides have no specific effect on dioxin solubility, other noncausal mechanisms are plausible. For example, the metabolic impairment that causes increased levels of triglycerides, insulin, and glucose could also increase the half life of dioxin. In the setting of such reverse causality, one might focus on diabetes preceding the elevated dioxin level as evidence in support of the (reverse causality) mechanism. But our data are not especially informative on this question, as the dioxin levels were measured at only one point. Diabetes diagnosed before 1987, for example, could be due to a relatively high background dioxin level in the distant past, and for a given subject the 1987 dioxin level may still reflect the previously "high" level. More than one of the causal scenarios just considered may be operating simultaneously. Because the levels of dioxin considered here are so low it is tempting to think that the solubility or half-life scenario accounts entirely for the observed relations; but whether it does cannot be determined from these data.

Plausible biologic mechanisms exist by which dioxin could impair insulin-glucose metabolism in humans. Enan and colleagues have shown that dioxin decreases glucose uptake in *in vitro* and *in vivo* models[3-5], and that the activity appears to be Ah-receptor mediated. Although Enan et al. have shown a clear dose-response effect of dioxin on glucose uptake *in vitro* using human cells[3], the lowest concentration studied was 100-fold greater than that found in background-exposed humans. The decrease in glucose uptake is possibly due to dioxin altering insulin-recruitable glucose transporter activity[3]. These findings provide a mechanism for earlier observations that dioxin increases serum glucose in animals[6,7]. As an aside we note that an effect of dioxin on serum lipids, especially triglycerides, has been demonstrated in animals, and in some human studies, but this effect has been observed at relatively high doses of dioxin and may not be linked to insulin-glucose metabolism.

In summary, the association of background-level dioxin exposure with the prevalence of diabetes in these data may well be due to reasons other than causality, though a causal contribution cannot be wholly dismissed. Pharmacokinetic and other experimental studies may be of particular use in assessing whether the association is accounted for by noncausal phenomena.

References

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