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The Adipose Tissue to Serum Dichlorodiphenyldichloroethane (DDE) Ratio: Some Methodological Considerations[1]

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Dichlorodiphenyldichloroethane (DDE) adipose tissue level has been regarded as a preferred indicator of accumulated human exposure to DDT; however, blood sera are more feasible to obtain and analyze than adipose tissue samples. Inconsistent and scarce information exists in relation to the adipose tissue/serum DDE ratio. As a part of a hospital-based case–control study performed in Mexico City from 1994 to 1996, 198 paired serum and adipose tissue samples were obtained from 72 women with histologically confirmed breast cancer and 126 women with benign breast disease. Both adipose tissue and serum DDE levels were determined by gas–liquid chromatography and reported as ppb lipid weight (ng/g) as well as wet basis (ng/ml). Results showed that the adipose tissue/serum DDE ratio (ADSE) varies according to the type of information (lipid vs wet basis, arithmetic vs geometric means) used for its estimation. ADSE gets a value near 1 (1.1) only when the geometric DDE levels in lipid basis are used for its estimation. The correlation between DDE serum and adipose tissue levels was found ($r = 0.364$, $P < 0.001$). The ADSE did not vary by disease status, nor was it altered by parity, history of breast-feeding, and other reproductive characteristics. We endorse the use of venipuncture instead of biopsy as a way to estimate DDT body burden levels in further research.

Key Words: **adipose tissue; blood serum; organochlorine compounds; DDT; reproductive characteristics.**

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INTRODUCTION

Human health impact due to environmental dichlorodiphenyltrichloroethane (DDT) chronic exposure is a topic of great interest (Safe, 1997). In the evaluation of human exposure, the dichlorodiphenyldichloroethane (DDE) adipose tissue level has been regarded as a preferred indicator (Anderson, 1985). However, due to ethical constraints, it is not always possible to obtain adipose tissue samples from healthy individuals to study the body burden of DDT. In addition, biopsies are more difficult to obtain and to manipulate and more expensive to analyze than serum, urine, or breast milk samples (Woodruff *et al.,* 1994).

A commonly employed alternative to measurement in adipose tissue, particularly for epidemiological studies, is the assessment of DDE levels in serum samples. Unfortunately inconsistent information exists regarding the relation between adipose and serum levels. The adipose tissue/serum DDE ratio (ADSER) has varied from 1:1 (Needahm *et al.,* 1990) to 200–1000:1 (Krieger *et al.,* 1994; Toppari *et al.,* 1996).

In this paper, we report the results of an assessment of the adipose tissue and serum levels using data from a study of DDT exposure and breast cancer among Mexican women (López-Carrillo *et al.,* 1997) to evaluate variation in the ADSER DDE ratio by individual characteristics.

MATERIALS AND METHODS

The study population consisted of 198 women that were recruited as part of a hospital-based case–control study that was performed in Mexico City from 1994 to 1996 (López-Carrillo *et al.,* 1997). Breast cancer

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was histologically confirmed in 72 of them, whereas 126 had benign breast disease. Serum and adipose tissue samples were obtained from nonfasting participating women during their diagnostic workup, but before any treatment.

Reproductive and socioeconomic characteristics of the women were obtained by personal interviews. Detailed information about the procedures to obtain the blood samples has been published elsewhere (López-Carrillo *et al.,* 1997). Adipose tissue samples (0.5 to 5 g) were obtained from the breast adipose tissue that was the residue of the diagnostic (excisional biopsy) or surgical maneuvers (mastectomy). Adipose tissue samples were collected into glass vials on ice and frozen at −70°C.

Serum and adipose tissue DDE levels were measured by means of gas–liquid chromatography, following the protocol recommended by the United States Environmental Protection Agency (U.S. Environmental Protection Agency, 1980). Total lipids in serum and adipose tissue samples were determined according to the method proposed by Akins *et al.* (1989). Concentrations were reported on the basis of ppb lipid weight (ng/g) as well as wet basis (ng/ml).

Results for 198 quality control samples (i.e., bovine serum fortified with DDE) showed a 94.6 ± 9.8% of recovery. In addition, each human serum sample was fortified with aldrin with an average recovery of 96.0 ± 11.5%. For quality control among adipose tissue samples 198 chicken fat samples were fortified with DDE with a percentage of recovery of 94.5 ± 9.9%, also each adipose tissue sample was fortified with aldrin yielding 93.0 ± 9.8% of recovery. The detection limit for DDE was 2 ng/g.

Arithmetic and geometric means (i.e., natural logarithm) were calculated for the adipose tissue and serum DDE levels, using the corresponding concentrations in lipid or wet basis. ADSER was the average of all individual adipose: serum ratios. The association between DDE in adipose tissue and DDE in serum was estimated by means of the Spearman correlation coefficient and linear regression. Analysis of variance was used to compare the ADSER throughout the study groups. All these analyses were performed with the statistical software STATA 4.0.

RESULTS

The average age of women who provided the biological samples was 40 years (range 19–78 years). One-third were nulliparous at the time of the interview and about two-thirds had 8 years of education (data not shown).

As shown in the Table 1, the adipose tissue/serum DDE ratio estimated from the arithmetic means was 161.3 and was not significantly higher among BC cases (170.9) than among BBD patients (144.6). The adipose tissue/serum DDE ratio estimated from the geometric means was 5.8 and was also similar for women with BC (4.6) and women with BBD (6.5).

Adipose tissue/serum DDE ratios estimated using the concentrations in lipid basis were lower than those estimated in wet basis. The adipose tissue/serum DDE ratio based on the arithmetic means was about 4.0 in the total study population and was not statistically different by disease status (5.6 vs 3.5). A ratio near the unity (1.1) was estimated for the relationship between adipose tissue DDE and serum

TABLE 1
Adipose Tissue/Serum Dichlorodiphenyldichloroethane (DDE) Ratios

	Breast cancer cases (n=72)			Benign breast disease (n=126)			All (n=198)		
	Adipose tissue	Serum	Ratio	Adipose tissue	Serum	Ratio	Adipose tissue	Serum	Ratio
Wet basis (ng/ml)									
Arithmetic mean	754.9	6.0	144.6	377.9	3.0	170.9	515.0	4.1	161.3
Min–max	24–5613.8	0.5–35.4	9.8–913.2	1.2–2425.9	0.2–30.6	0.41–2291.7	1.2–5613.8	0.2–35.4	0.41–2291.7
Geometric mean[a]	396.3	4.10	4.6	218.3	2.2	6.5	270.1	2.8	5.8[b]
Min–max	24–5613.8	0.5–35.4	−22.8, 41.6	1.2–2425.9	0.2–30.6	−150.4, 109.6	1.2–5613.8	0.2–35.4	−150.4, 109.6
Lipid basis (ng/g)									
Arithmetic mean	1572.5	785.77	5.6	712.23	388.9	3.5	1025.1	532.2	4.2
Min–max	45.5–17387	10.2–4661.4	0.1–88.5	16.8–7271.5	20.9–2572.3	0.05–65.3	16.8–17387	10.2–4661.4	0.05–88.5
Geometric mean[a]	738.9	424.6	1.1	393.30	272.2	1.1	492.7	319.9	1.1[c]
Min–max	45.5–17387	10.2–4661.4	0.7–2.9	16.8–7271.5	20.9–2572.3	0.5–2.1	16.8–17387	10.2–4661.4	0.5–2.9

[a] Values were antilog transformed
[b] ln DDE serum=−0.95+0.351 ln DDE adipose tissue, R^2=24.50, Spearman coef.=0.494 ($P<0.001$) n=198, wet basis.
[c] ln DDE serum=−3.80+0.318 ln DDE adipose tissue, R^2=12.03, Spearman coef.=0.364 ($P<0.001$) n=198, lipid basis.

DDE, when adjusting for total lipid content and using the geometric means.

The Spearman correlation coefficients between the geometric means of DDE in serum and adipose tissue samples, in wet and lipid bases, were 0.494 and 0.364, respectively, and were statistically significant. For each ppb of DDE in serum, we calculated that 0.351 ppb of DDE will be present in the adipose tissue on wet basis and 0.318 ppb of DDE in lipid basis.

Selected characteristics of the women in relation to the ADSER are shown in the Table 2. The ADSER was at the borderline of significance among older women (>45 years) and it was statistically significant for menopausal women compared to younger and premenopausal women, respectively (ADSER = 1.09 for age < 35 vs 1.18 for age >45, and 1.07 for premenopausal vs 1.20 for postmenopausal). The ADSER did not vary significantly by parity, age at first birth, breast-feeding history, Quetelet index, or disease status.

TABLE 2
Selected Characteristics in Relation to Adipose Tissue/Serum Dichlorodiphenyldichloroethane (DDE) Ratio[a]

Variables	χ	95% CI	(*n*)
Age (years)			
<35	1.09	1.04–1.15	(85)
35–45	1.04	0.99–1.10	(54)
>45	1.18*	1.08–1.29	(59)
Parity			
⩾4	1.12	1.03–1.21	(46)
1–3	1.09	1.03–1.14	(98)
Nulliparous	1.13	1.05–1.22	(54)
Age at first birth			
<20	1.10	1.01–1.19	(47)
20–24	1.13	1.05–1.21	(60)
⩾25	1.04	0.96–1.12	(37)
Brest-feeding at first birth (months)			
0	1.10	1.03–1.17	(40)
1–6	1.06	1.00–1.13	(53)
7–12	1.16	1.03–1.28	(37)
>12	1.06	0.85–1.26	(14)
Breast-feeding all births (months)			
0	1.08	1.00–1.17	(28)
1–6	1.04	0.97–1.12	(27)
7–12	1.05	0.95–1.16	(27)
>12	1.15	1.06–1.24	(62)
Menopausal status			
No	1.07	1.04–1.11	(143)
Yes	1.19**	1.08–1.31	(55)
Quetelet index			
<24	1.08	1.03–1.14	(103)
25–29	1.18	1.10–1.27	(66)
⩾30	1.02	0.98–1.07	(29)
Disease status			
Breast cancer	1.14	1.06–1.22	(72)
Benign disease	1.09	1.04–1.13	(126)

[a] DDE geometric mean, lipid basis.
*ANOVA, P=0.046.
** t test, $P<0.05$.

DISCUSSION

Our results show that the adipose tissue/serum DDE ratio varies according to the method of expression. The ratio is near 1 when serum levels are expressed as geometric means on a lipid basis. Ratios are larger for arithmetic means and lipid basis (4 or 5), geometric means on a wet basis (4 or 5), and arithmetic mean on a wet basis (about 150). The ratios did not vary by disease status which suggests that breast cancer itself does not have much of an impact in serum levels.

Some of the discrepancies found in the literature (Kanja *et al.*, 1992; Krieger *et al.,* 1994) might be explained by the different type (lipid or wet basis; arithmetic or geometric means) of DDE adipose/tissue concentrations used for particular studies.

The biological samples in this study were obtained from nonfasting women. Some studies have pointed out that lipid adjustment is not needed when fasting samples are being analyzed. Hence, the difference between the values of DDE in wet and lipid bases that have been found in this and previous studies might be also partially explained by the characteristics of the studied population (fasting vs nonfasting) (Phillips *et al.,* 1989).

The significant correlation coefficient found between DDE serum and breast adipose tissue observed here is consistent with previous studies that have reported a significant correlation between DDE serum levels and subcutaneous fat (Mussalo-Rauhamaa, 1992; Kanja, 1992) and breast adipose tissue (Archibeque-Engle *et al.,* 1997).

Except for a slightly larger ratio among older women, the relationship between serum and adipose levels did not show much variation by individual characteristics including parity, age at first birth, breast-feeding, menopausal status, or Quetelet index. This indicates serum levels of DDE are a reasonable reflection of adipose concentrations over a wide range of situations.

CONCLUSIONS

We conclude that serum samples can be confidently used in research as a most practical means to calculate DDT human body burden levels. This is important because venipuncture is less invasive method than biopsy for obtaining samples, and the chemical analytical procedures for serum analysis are less expensive than those required for adipose tissue.

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