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Comparison of Organochlorine Pesticide Levels Between Abdominal and Breast Adipose Tissue

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The contamination of humans with persistent organochlorine pesticides is caused by a combination of their retarded degradation rates in the environment, soil deposits and historically extensive use (Harris et al 2000). The benefits of their use in malaria combat (WHO 1973) have raised concerns due to their stability, ubiquitous presence and persistence in the environment, accumulation in adipose tissue and estrogenic properties (Waliszewski et al 1998, Turusov et al 2002).

Because of DDT's volatility and widespread propagation, the main route of human exposure in tropical areas consists of inhalation of contaminated air (Jury et al 1982, Mathies et al 1991). An alternative route of human exposure consists of consumption of contaminated food, especially of animal origin (Waliszewski et al 1995, 1998). Organochlorine pesticides, caused by their lipophilic nature and high persistence, accumulate in food chains and in the human body, especially in lipid-rich tissues, such as adipose tissue.

Assimilation of these compounds through the diet and inhaling air, due to their antiandrogenic and estrogenic properties and effects on sexual hormones activity, may play a role in the etiology of human breast cancer (Wolff 1995, Alhorg et al 1995, Kang et al 1996, Guttes et al 1998, Golden et al 1998, Strucinski 2001). Thus, the monitoring of human tissues could serve as an indicator for understanding the biological specificity in the accumulation of organochlorine pesticides, as well as for assessing their participation in environmental pollution. The study to determine the significance of higher residue levels and their influence on human affections can be achieved only when the results are comparable statistically to the others obtained from control groups. The aim of the present study is to compare the levels of persistent organochlorine pesticides between breast adipose tissue and abdominal adipose tissue. The samples were obtained from women without any breast diseases, submitted to autopsy at the Institute of Forensic Medicine in Veracruz, deceased during automobile accidents. The pesticide levels are statistically compared to calculate the possible differences between both

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sample groups and to establish if abdominal tissue can serve as an adequate control sample for the breast cancer study.

MATERIALS AND METHODS

Adipose tissue (5 grams) from abdominal cavities and breasts without pathology from 60 randomly selected women were obtained during autopsy done at the Institute of Forensic Medicine of University of Veracruz. The adipose tissue was stored in pretreated glass jars, immediately frozen and kept at –25°C until analyzed.

Adipose tissue samples were analyzed according to the previously described method (Waliszewski and Szymczynski 1982). The qualitative and quantitative determinations were done by gas chromatography on a Varian 3800 apparatus equipped with a ^{63}Ni electron capture detector. For pesticide separation, a fused silica column SPB 608 30 m x 0.32 mm ID, 0.5 µm film was used at the following temperature program: 193°C (for 7 min) to 250°C at 6°C/min, hold 20 min. The carrier gas was nitrogen at 2.3 ml/min, and split/splitless sample injection of 1 µl was employed.

All samples were analyzed for HCB, β-HCH, pp'DDE, op'DDT and pp'DDT. The minimum detection limits for the organochlorine pesticides studied in adipose tissue were 0.001 mg/kg for HCB and β-HCH, 0.002 mg/kg for pp'DDE, op'DDT and pp'DDT. To determine the quality of the method, the recovery study was performed on ten overspiked replicates of a blank cow fat sample, which revealed contamination levels below detection limits. The fortification study, done at 0.010 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 89 to 95% of recovery. The standard deviation and coefficients of variation were below 8, indicating excellent repeatability of the method. The concentrated sulfuric acid used in the clean-up step of adipose tissue extracts, permits quantitative fat precipitation and degrades the ubiquitous phthalate esters that interfere with the gas chromatographic identification of organochlorine pesticides (Waliszewski and Szymczynski 1990).

Organochlorine pesticide residue mean values in abdominal adipose tissue and breast adipose tissue were calculated using basic statistics. To compare variability between abdominal adipose tissue and breast adipose tissue concentrations, paired T-Test, the Pearson correlation coefficients (r), linear regression coefficients (β), Mann-Whitney test and differences of ANOVA, F-test and two-tailed t test to determine tail probability were calculated using the statistical software Minitab version 12.

RESULTS AND DISCUSSION

The chemical equilibrium for deposition of persistent organochlorine pesticides in the human body considers the internal transport and

equilibrium pattern between the pesticide concentrations in adipose tissue and blood serum. This model describes the internal distribution of lipophilic compounds (Parham et al. 1997). The equilibrium of contents between compartments can be defined as chemical fugacities between adipose tissue (deposit compartment) and blood lipids (transport compartment) (Noren et al. 1999). The movement of persistent organochlorine pesticides across biological membranes occurs bidirectionally and the mode of membrane passage is applicable in both directions (Waliszewski et al. 2001). The liposolubility is a major factor that influences the rate of accumulation and elimination from tissues and organs (Brown and Lawton 1984). Thus, it can be supposed that apparently equal concentrations exist in lipid rich compartments of the organism and the existing differences depend principally on the lipid content of the tissues (Henriksen et al. 1998, Russell et al. 1999).

Adipose tissue from abdominal cavities and from breasts was taken to determine the organochlorine pesticide residue concentrations in both paired samples. The adipose tissue was taken from the same 60 women during autopsies who had no breast diseases. The results obtained as mg/kg on fat basis are presented in Table 1 as mean values (x), standard deviation of means (SD), medians, ranges and 95% of confidence intervals (CI). The comparison of mean values and standard deviations which provide measures of how spread out the data are for all organochlorine pesticides between abdomen and breast adipose tissues indicates no significant differences between both sample groups. The same pattern follows medians, ranges and 95% of confidential intervals.

Table 1. Means (x), standard deviations of means (SD), medians, ranges and 95% CI of persistent organochlorine pesticide levels (mg/kg) between feminine adipose tissue from abdomens ((abd) and breasts (br), (n=60).

Pesticide	x ± SD	Median	Ranges	95%CI
HCB abd	0.055 ± 0.041	0.045	0.009 - 0.202	0.042, 0.069
HCB br	0.057 ± 0.047	0.045	0.013 - 0.218	0.042, 0.072
β-HCH abd	0.392 ± 0.254	0.317	0.011 - 0.194	0.309, 0.474
β-HCH br	0.389 ± 0.256	0.342	0.020 - 0.176	0.307, 0.472
pp'DDE abd	1.220 ± 0.612	1.122	0.370 - 3.104	1.022, 1.418
pp'DDE br	1.170 ± 0.588	1.061	0.364 - 2.873	0.980, 1.361
op'DDT abd	0.074 ± 0.078	0.050	0.006 - 0.031	0.049, 0.099
op'DDT br	0.077 ± 0.088	0.046	0.006 - 0.029	0.048, 0.105
pp'DDT abd	0.638 ± 0.428	0.576	0.017 - 0.295	0.499, 0.777
pp'DDT br	0.624 ± 0.428	0.546	0.015 - 0.294	0.485, 0.762
Σ-DDT abd	1.949 ± 0.885	1.906	0.527 - 1.177	1.662, 2.237
Σ-DDT br	1.871 ± 0.950	1.709	0.392 - 1.164	1.563, 2.179

The analyses of paired groups for organochlorine pesticide contents in abdominal and breast adipose tissue are shown in Table 2 as the Pearson correlation coefficients (r), regression coefficients (β) to express the magnitude of correlation and the equality of two population medians with Mann-Whitney test. The obtained results of correlation indicates the coefficients 0.926 for HCB, 0.958 for β-HCH, 0.842 for pp'DDE, 0.987 for op'DDT, 0.947 for pp'DDT and 0.886 for Σ-DDT. All are near to one, indicating significant correlation of organochlorine pesticide concentrations in both sample groups. The same order follows the regression coefficients confirming the previous conclusion of no differences between organochlorine pesticide concentrations in adipose tissue from abdomens and breasts. Calculated p value for all paired group showed <0.0001 value. Thus, the pairing results indicate a significant correlation between both columns. The applied Mann-Whitney test, to compute the equality of two population medians from independent random samples that have the same shape, reveals its significance for all paired pesticides and samples and confirms the equal distribution of organochlorine pesticide contents between abdominal and breast adipose tissue.

Table 2. Pearson correlation coefficients (r), regression coefficients (β) and Mann-Whitney test of organochlorine pesticide levels (mg/kg) between feminine adipose tissue from the abdomen and breast.

Pesticide	r	β	Mann-Whitney
HCB	0.926	0.812	0.795
β-HCH	0.958	0.951	0.968
pp'DDE	0.842	0.875	0.723
op'DDT	0.987	0.875	0.956
pp'DDT	0.947	0.948	0.889
Σ-DDT	0.886	0.826	0.562

To compare the variance differences between both paired groups, the ANOVA and F-tests were applied. The obtained results of one-way analysis of variance ANOVA are low or negative and F-test are less than 1 indicating similarity of variances for both sample groups. The F-test results are significantly less than 1, which shows that the variances and standard deviations of paired abdominal and breast organochlorine pesticide concentrations are statistically equal and indicate no differences between means of their concentrations. Furthermore, the results indicate that the data are unlikely to be sampled from populations with equal means and that the standard deviations are equal, which can be corroborated in table 1. Effective pairing results of t-test and two-tailed p value indicate a significant correlation and no differences between means and no significant differences between variances among the columns of

organochlorine pesticide concentrations in abdominal and breast adipose tissue.

Table 3. One-way analysis of variance (ANOVA), F- test and paired t test applied to determine differences between means in organochlorine pesticide levels (mg/kg) between adipose tissue from the abdomen and breast.

Pesticide	Differences ANOVA	F	t	two-tailed p value
HCB	- 0.0013	0.4207	0.4514	0.6543
β-HCH	0.0020	0.9633	0.1669	0.8684
pp'DDE	0.0195	0.8132	0.9133	0.3669
op'DDT	- 0.0028	0.4591	1.0760	0.2887
pp'DDT	0.0147	0.9959	0.6574	0.5149
Σ-DDT	0.0786	0.6673	1.1100	0.2738

In conclusion, it can be expressed that a good correlation exists between concentrations of persistent organochlorine pesticides in abdominal and breast adipose tissue and a uniform distribution of these compounds in adipose tissue in both compartments of the human body. Therefore, the abdominal adipose tissue from healthy patients can serve as the control sample. The sample can be compared to the breast cancer patients in the study to see, if statistical differences of xenoestrogen concentrations really exist and if these levels can correlate with development of breast cancer in women.

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