



Associations between Obesity, Body Fat Distribution, Weight Loss and Weight Cycling on Serum Pesticide Concentrations

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Abstract

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Objective: Preliminary studies suggest pesticides may be linked to increased cancer risk. Since most pesticides are lipophilic and stored within adipose tissue, serum levels of organochlorines are affected not only by environmental exposures, but also by factors related to lipid turnover and storage. Our objective was to investigate whether serum organochlorines are influenced by weight loss, body fat distribution, and weight cycling.

Methods: Ten overweight women were recruited upon entry into a weight loss program and surveyed regarding weight history, childbearing/lactation, and exposure to environmental contaminants. Anthropometric measures and phlebotomy were conducted at baseline and at four weeks (mean weight loss=5.1 kg). Serum was analyzed for 19 common polychlorinated pesticides and metabolites and 10 PCB congeners.

Results: Organochlorine levels were not significantly affected by weight loss nor associated with body mass index (BMI). Strong positive correlations were noted between levels of DDE/DDT and age (DDE $\beta=0.6986/p=0.0246$ /DDT $\beta=0.6536/p=0.0404$) and between DDE/DDT and waist-to-hip ratio (WHR) (DDE $\beta=0.4356/p=0.0447$ /DDT $\beta=0.8108/p=0.0044$). Trends were noted for decreased levels of DDT in women who reported more episodes of weight cycling.

Conclusion: Serum organochlorine levels may be affected not only by age, but also factors related to lipid turnover (i.e., episodes of weight cycling and WHR), and warrants further study.

Keywords: Organochlorines; Body fat; Waist hip ratio; Weight cycling; Weight loss; Obesity

Introduction

The incidence of breast cancer has increased markedly since the 1940s, as have environmental factors that are implicated as potential contributors to risk [1]. Exploration of a class of environmental contaminants, known as organochlorines, and their associations with hormonally-linked cancers has been cited as an area of interest [2,3].

Organochlorines are primarily composed of two families of compounds, chlorinated pesticides (DDT, its metabolite DDE, and related compounds) and polychlorinated biphenyls (PCB), comprised of 209 PCB congeners. Rat models were first used to show that organochlorines stimulate the growth of mammary tumors [4,5]. Parallel research in humans found the following: 1) Dewailley et al. found women with high levels of serum DDT had four times the risk of estrogen receptor positive breast cancer when compared to women with lower levels [6]; 2) Falck et al. found that levels of PCBs in mammary tissue were significantly greater in 23 cases than 27 controls [7]; and 3) Wolff et al. found that serum levels of DDE were significantly elevated in breast cancer cases in a nested case-control study [8]. In contrast, a study by Krieger et al. with prospectively collected blood samples, found no differences in serum levels of DDE or PCBs among a sample of 150 women who developed breast cancer versus a sample of 150 who did not [9]. However, when women were divided into racial subgroups, chemical exposure continued to be non-significant between White cases and controls, but was a factor for Blacks. In addition, more recent studies determining adipose concentrations of organochlorines in women found that Black women typically presented with significantly greater concentrations of DDE and PCB congeners than their White counterparts [10,11]. Women with high concentrations of organochlorines, therefore may be a population who may be at risk of breast cancer, thus the determination of preventive measures to improve their outcomes is necessary.

Since organochlorines are stable, lipophilic compounds that are largely stored in adipocytes, a potential exists for environmental exposures to be modified by adipose tissue or factors related to lipid mobilization. An examination of a nationally representative sample of United States adults indicated that PCBs and other persistent organic pollutants were higher in women over 40 years old and positively correlated with trunk fat [12]. A meta-analysis of case-control studies analyzing the relationship between DDT/DDE with breast cancer found no overall association between DDT exposure and breast cancer, but only observed increased risk with higher serum DDE (OR=1.15, 95% CI [1.01-1.32]) [13]. Another recent meta-analysis found no association between DDE exposure and increased breast cancer risk [14], but neither analyses addressed the potential confounding of adiposity which is key since consensus exists regarding the association between obesity and post-menopausal breast cancer [15-19]. Several studies have found positive associations between the disease, waist-to-hip ratio, and weight cycling, which may serve as indicators of lipid turnover [20,21].

We present results from an exploratory study that was undertaken to investigate whether weight reduction resulted in changes in levels of serum organochlorines or if other factors related to lipid mobilization (i.e., body fat distribution and history of weight cycling) were

associated with baseline organochlorine levels in ten normal overweight women enrolled in a structured weight reduction program.

Methods

Study subjects were recruited from new participants enrolled in the Duke University Diet and Fitness Center (DFC) structured, one-month intensive weight loss program. The multidisciplinary approach at DFC incorporates an individualized very low calorie diet into lifestyle education and training using behavior modification and physical activity to support rapid weight loss as well as long-term weight maintenance [22]. Institutional Review Board approval was granted and written consent was obtained from all ten women. A baseline survey eliciting information related to weight history and pesticide/herbicide/fungicide exposure was administered and fully completed by nine subjects. Weight, height, and waist and hip circumference were taken before the subjects began the weight loss program and after 4 weeks.

Circumferences were measured with a non-stretch tape measure placed at horizontal planes of the maximal extension of the buttocks for hip circumference and one inch about the umbilicus for waist circumference. Anthropometric measures were taken with subjects standing in their underclothes. Measures were recorded to the nearest tenth of a kilogram for weight and to the nearest tenth of a centimeter for other measures.

Twenty milliliters of blood was drawn from fasted subjects at baseline and at four week follow-up. Serum was separated from whole blood and stored in Teflon-topped tubes at -40°C. Frozen baseline and follow-up samples were then batched-shipped on dry ice for determination of organochlorines and total lipid levels (AccuChem Laboratories, Richardson, TX).

Organochlorine analyses were conducted via high-resolution gas chromatography with electron capture using a Hewlett Packard 5890 GC equipped with a Supelco SPB-608 fused silica capillary column (30 m x 0.025 id). Sera was analyzed for chlorinated pesticides (α and γ chlordane, oxychlordane, heptachlor, heptachlor epoxide, trans-nonachlor, α , β , δ and γ benzene hexachloride, aldrin, endrin, dieldrin, hexachlorobenzene endosulfans I and II, DDE, DDD and DDT) and major polychlorinated biphenyl congeners (2,3', 4,4' tetra; 2,3', 4,4', 5 penta; 2,2', 4,4', 5,5' hexa; 2,3,3', 4,4' penta; 2,2'3, 4,4', 5,5' hexa; 2,2', 3, 4,4', 5' hexa; 2,2', 3, 4,4', 5', 6 hepta; 2,2', 3,3', 4,4', 6 hepta; 2,2', 3, 4,4', 5,5' hepta; 2,2', 3,3', 4,4', 5 hepta and total PCBs). The detectable limit for all compounds was <0.3 ng/ml or ppb. Due to the lipophilic nature of organochlorines, lipid adjustment is suggested [23]. Analysis for total lipid was conducted by a gravimetric procedure. Lipids were extracted by the method of Folch et al. as modified by Ulman and McCluer using chloroform/methanol (2:1) as the initial solvent [24,25]. Reliability was tested using inter- and intra-assay precision checks. External controls constituted 10% of the batch sample. Final concentrations of organochlorines were expressed in terms of lipid-adjusted levels, as suggested by Phillips et al. [23].

Data were double-keyed and verified. Organochlorine levels that registered <0.3 ng/ml or "undetectable" were assigned a value of 0.15 ng/ml for purposes of analyses. This value was chosen since it represents the midpoint between a true zero reading, which is improbable, and the detectable limit of 0.3 ng/ml and therefore considered a conservative estimate. Data analyses were conducted

using SAS version 6.08 for Windows (Cary, NC). Pearson correlation coefficients were generated to explore potential associations between levels of organochlorines and continuous variables at baseline. Associations between DDE/DDT levels and waist-to-hip ratio were further explored using multiple linear regressions adjusted for age. T-tests were employed to test for differences in baseline levels of organochlorines by variables which were categorical and could be dichotomized. Differences between levels of organochlorines at baseline and after four weeks on the weight reduction program were tested using t-tests.

Results and Discussion

The ten women participating in this investigation were Non-Hispanic Whites between the ages of 34 and 67 years. All women resided in urban or suburban areas with most from major metropolitan centers. All women were overweight or obese, with BMIs ranging from 28.5 to 46.8 kg/m² ($x=36.6 \pm 6.4$). One subject discontinued participation in the study soon after accrual and therefore the sample size for some analyses (i.e., those related to weight loss) was nine instead of ten. Mean weight loss during the 4 week program was 5.07 ± 1.87 kg.

Although serum was tested for 29 chlorinated pesticides and PCBs, many of these compounds were undetectable in either the serum collected at baseline or follow-up. Compounds that were undetectable in all subjects throughout the study were α and γ chlordane, δ and γ benzene hexachloride, aldrin, endrin, heptachlor, endosulfans I and II, DDD and 2,3', 4,4' tetra; 2,3,3', 4,4' penta; 2,2', 3,4,4',5 hexa 2,2',3,4,4',5',6 hepta; 2,2',3,3',4,4',6 hepta and 2,2',3,3',4,4',5 hepta PCBs. Data analyses were limited to DDE and total PCBs, which were present in detectable levels in all ten subjects, and DDT, which was detectable in five.

Little variation in response to survey questions regarding exposure to contaminants was noted; therefore, further efforts to correlate self-reported exposure to serum markers were halted. Variation with fairly normal distribution was noted for data representing BMI (weight (kg)/height (m²)), age, and waist-to-hip ratio. In addition, the sample could be dichotomized into women who repeatedly weight cycled (lost and gained at least 15 pounds at least four times during their lifetime, n=3) versus those who did not (n=6). Therefore, analyses focused on this factor, in addition to testing for potential associations between weight reduction and serum organochlorine levels.

Pearson correlation coefficients and associated p-values between specific organochlorines and age, BMI, and waist-to-hip ratio are presented in Table 1. There was little association between organochlorine levels and BMI. Likewise, there appeared to be little association between total PCBs and age and between total PCBs and waist-to-hip ratio. However, for both DDE and DDT, there was a strong and significant positive association with both age and waist-to-hip ratio. Since waist-to-hip ratio and age are correlated, the relationship between waist-to-hip ratio and DDE and DDT were explored further. After adjusting for age, the association between waist-to-hip ratio and DDT levels remained significant ($p=0.0482$). In contrast, when the effect of age was controlled for the significance of the association between DDE and waist-to-hip ratio diminished. These findings are consistent with a previous study's finding of significant correlations between organochlorine pesticides, BMI, and waist-to-hip ratio in urban Chinese women [26].

Compound	BMI ^a		Age ^b		Waist-to-hip Ratio ^c	
	Correlation	p-value	Correlation	p-value	Correlation	p-value
PCBs:						
Total PCBs	-0.2170	0.5470	-0.2439	0.4972	0.1854	0.6081
Chlorinated Pesticides						
DDE	0.5430	0.8816	0.6986	0.0246**	0.4356	0.0447**
DDT	0.2152	0.5506	0.6536	0.0404**	0.8108	0.0044***
^a BMI = MEAN (SD) = 36.6 (6.4), range = 28.5-46.8 ^b Age =MEAN (SD) = 45.3 (12 years), range = 34-67 ^c Waist-to-hip Ratio = MEAN (SD) = 0.88 (0.09), range = 0.77-1.03 ** significant p<0.05 *** significant p<0.01						

Table 1: Correlations between serum levels of lipid adjusted organochlorines and body mass index, age, and waist-to-hip ratio at baseline.

Organochlorine levels of dichotomized women who chronically weight cycled versus those who did not are presented in Table 2. Women who reported at least four episodes of losing and gaining 15 pounds during their lifetime generally had lower levels of serum organochlorines than those who reported three or less episodes.

Differences approached significance for total PCBs and DDT. Although, there was a trend toward lower total PCB levels upon weight loss, there were no significant differences between levels of DDT, DDE, or PCB in sera collected at baseline versus that collected at study completion.

Compound	Number of Episodes Women Lost or Gained > 15 pounds		
	≤ 3(n=7) MEAN (SD)	≥ 4(n=3) MEAN (SD)	p value
PCBs			
Total PCBs	233 (155)	103 (53)	0.0842*
Chlorinated Pesticides			
DDE	816 (932)	430 (140)	0.3226
DDT	43 (25)	23 (22)	0.0812*
*trend, p<0.1			

Table 2: Mean levels of serum lipid-adjusted organochlorines of women dichotomized into groups with/and without history of weight cycling.

This investigation was driven by the hypothesis that organochlorines (stable lipophilic compounds that are stored in adipose tissue) would be liberated into the serum, thus increasing in concentration upon weight loss. Parallel research in polar bears, bats, mice, and cockerels suggests that serum levels of DDT/DDE and/or PCBs increase upon starvation and energy restriction [3,5,27-29]. A study conducted in males and females who underwent prolonged weight loss also found results of elevated organochlorine pollutants in both plasma and subcutaneous adipose tissue [30]; however, results of this study do not support this previous work since serum levels for most compounds studied either remained the same or decreased with weight loss (total PCBs). Potential explanations for our study results may be ascribed to the following reasons: 1) previous episodes of weight loss in this sample of women may have already cleansed their fat stores; 2) the study period was too brief to detect changes that may ultimately occur; and/or 3) organochlorines may have been

redistributed to other tissues with initial weight loss and thus changes in serum levels may not be detectable.

Analyses of baseline data suggest that women who weight cycled four or more times during their lifetime had lower serum organochlorine levels than women who reported fewer episodes. Further study is needed to determine if the effects of weight cycling result in relocation or elimination of organochlorine compounds to determine if, as this study suggests, serum levels of organochlorines decrease with weight loss. What these changes in serum levels imply about redistribution of persistent lipophilic substances from fat to other target tissues of concern (breast, uterus, liver, etc.) remains a key unresolved issue.

There was a strong positive association between levels of DDE and DDT, and both age and waist-to-hip ratio [1,31]. Since DDT has been banned from the United States for many decades, it is not surprising

that older women have higher levels of both DDT and its metabolite DDE. These findings support studies, such as the Environmental Protection Agency's National Adipose Tissue Survey, which show that environmental contaminants that are stable and lipophilic bioaccumulate with age [32].

The strong positive association between levels of DDE/DDT and waist-to-hip circumference may be attributed to at least two phenomena. First, waist-to-hip ratios are in themselves positively associated with age and may serve as an artifact of aging. Although the correlation between waist-to-hip ratio and levels of DDE were diminished when age was controlled for, the strong association with DDT continued to hold. This finding suggests that waist-to-hip ratio, perhaps because it signals the presence of more metabolically-active adipose tissue with greater rates of turnover, may be an important modifier of DDT exposure [33]. In conjunction with these findings, Stevens et al. also confirmed positive associations between waist circumference and DDT, emphasizing that environmental contaminants play an integrated role in the dynamics of weight control and further may contribute to excessive weight gain [34]. Indeed, waist-to-hip ratio has been positively associated with breast cancer, as well as other neoplasms [20,21]. Secondly, although the mechanisms underlying the contribution of waist-to-hip ratio to cancer risk are unknown at this time and speculation regarding the effect of fat distribution has been largely relegated to its interaction with hormonal levels, the relationship may be more complex and involve environmental contaminants as well, many of which also serve as xenoestrogens [32]. A comprehensive study of exposures to lipophilic environmental contaminants and factors potentially modifying their activity within the body, such as fat deposition is needed in the study of disease as complex as cancer. Such study may be beneficial in determining why racial subgroups (e.g., Blacks in the studies of Kreiger et al. and Schildkraut et al.) had larger differences in serum organochlorine levels between cases and controls, especially since past research suggests that Black women have greater waist-to-hip ratios [35]. This line of research may be particularly illuminating for triple negative breast cancer in which large disparities between Blacks and Whites are observed [36].

Conclusions

The study of organochlorines is extremely complex and findings of this exploratory study suggest that serum levels of DDT and DDE are affected by factors that govern their excretion, as well as factors related to lipid turnover (episodes of weight cycling and waist-to-hip ratio). In addition to age, which may serve as an index of cumulative exposure to these exceptionally stable compounds, factors associated with lipid mobilization may modify the exposure of specific target tissues to these compounds once in the body. The higher lipid turnover rate of adipose tissue in central body zones may modify tissue exposure to these potential carcinogens and thereby amplify risk. Findings of this investigation suggest a need for research that further explores the association between factors related to lipid mobilization/turnover and levels of environmental contaminants. Larger and more in-depth investigation may yield the discovery of interrelationships and further our knowledge of the potential causes and preventions of complex disease, such as breast cancer.

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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