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Chlorinated Hydrocarbon Levels in Human Serum: Effects of Fasting and Feeding

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Abstract. Twenty healthy adult humans had serum samples drawn on four occasions within a 24-hr period: after a 12 hr overnight fast, 4–5 hr after a high fat breakfast, at midafternoon, and the next morning after another 12 hr fast. Nonfasting samples had 22% to 29% higher mean concentrations ($p < 0.05$) than did fasting samples for polychlorinated biphenyls (PCBs, 4.81 vs 3.74 ng/g serum wt), hexachlorobenzene (HCB, 0.163 vs 0.134 ng/g serum wt), and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE, 6.74 vs 5.37 ng/g serum wt) measured by electron capture gas liquid chromatography. Total serum lipids were estimated from measurements of total cholesterol, free cholesterol, triglycerides, and phospholipids and were 20% higher in nonfasting samples than in fasting samples (7.05 g/L vs 5.86 g/L). When PCBs, HCB, and *p,p'*-DDE concentrations were corrected by total serum lipids, results from fasting and nonfasting samples were not statistically different. Because of the differences in these chlorinated hydrocarbon concentrations observed with different sample collection regimens, meaningful comparison of analytical results requires standardizing collection procedures or correcting by total serum lipid levels.

As a result of their widespread use, some chlorinated hydrocarbon compounds, such as polychlorinated biphenyls (PCBs) and many pesticides, are distributed worldwide as environmental pollutants and measurable levels are found in a considerable portion of the general population (Murphy and Harvey 1985; Safe 1984). These compounds are highly lipophilic and partition among various tissues depending on their lipid content (Morgan and Roan 1970; Matthews and Dedrick 1984). Total blood lipid levels increase following a fatty meal. For example, Cohn *et al.* (1988) fed adult humans 1.0 g of fat/kg of body weight and 7.0 mg/kg of cholesterol after a 14-hr fast. Three hr later, triglyceride levels had increased an average of 121% and total plasma lipids had increased 27%. Collection of serum samples for chlorinated hydrocarbon or other determinations is often, but not always, done in a fasting state because it is thought to be a more reproducible baseline (Cohn *et al.* 1988). Because chlorinated hydrocarbon concentrations partition according to the lipid content of tissues, and serum lipid levels in turn vary between fasting and postprandial states, this may lead to important differences in results depending on the specimen collection procedure.

The purposes of this study were (1) to determine the effect of fasting and serum lipid levels on serum chlorinated hydrocarbon concentrations, and (2) to determine if correction for serum lipid content removed any variation in chlorinated hydrocarbon concentrations between fasting and nonfasting specimens.

Methods

A 20 mL blood sample was taken from 20 adult human volunteers (10 males, 10 females, all Centers for Disease Control em-

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ployees) after a 12 hr overnight fast. The subjects were healthy and were not known to have been unusually exposed to the chlorinated hydrocarbon compounds of interest in this study. Following this sample, each adult ate a large triglyceride-rich breakfast consisting of three pieces of bacon, two patties of sausage, two eggs, toast with butter and jelly, grits with butter, and a piece of cheesecake. A second prelunch 20 mL blood sample was taken about noon, and then each person ate their regular lunch. A third 20 mL blood sample was drawn in midafternoon (3–4 pm) and a fourth 20 mL sample was taken in the morning before breakfast after a second 12 hr fast. This design permits evaluation of the effects of sample collection times on measured concentrations of selected chlorinated hydrocarbons, with each person serving as his/her own control. All blood samples clotted at room temperature for 30 minutes. The serum was separated by low speed centrifugation (2500 × g for 15 min) at 4°C and recovered by aspiration, and the samples were stored frozen at −20°C until analysis.

Three chlorinated hydrocarbon compounds were selected for analysis based on their high prevalence in previous Centers for Disease Control studies: hexachlorobenzene (HCB); *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), a metabolite of DDT; and PCBs. The analytical method involved extracting methanol-deproteinated serum with hexane and ethyl ether and eluting the organic extracts through deactivated silica gel (9 mm I.D. Chromaflex column with 50 mL reservoir) with hexane. The hexane fraction was analyzed using electron-capture (^{63}Ni) gas liquid chromatography and quantitated electronically by peak area (Burse *et al.* 1983) using decachlorobiphenyl as an internal standard. PCBs were quantitated as Aroclor® 1260. The CVs for the HCB, *p,p'*-DDE, and PCB assays at roughly normal serum concentration levels are 9.5%, 5.6%, and 8.9%, respectively.

Total cholesterol and triglyceride measurements were made on whole serum using standard enzymatic procedures on a DuPont ACA III analyzer[2] (DuPont Co., Wilmington, DE). Free cholesterol was determined enzymatically by the method of Cooper *et al.* (1982) using cholesterol oxidase in the absence of cholesterol esterase. Serum phospholipids were determined by the method of Takayama *et al.* (1977). Free cholesterol and phospholipid assays were automated by adapting them to an Abbott Super VP analyzer[2] (Abbott Laboratories, Irving, TX). All lipid analytical results represent the mean of duplicate determinations.

Estimates of total serum lipids were calculated by summation of the individual lipid components by the formula:

$$TL = 1.677\,(TC - FC) + FC + TG + PL \qquad (1)$$

where TL is total lipids, TC is total cholesterol, FC is free cholesterol, TG is triglycerides, and PL is phospholipids, all expressed in units of g/L. The first term in the equation represents cholesterol esters and is determined by multiplying the cholesterol component of the cholesterol ester fraction (TC − FC) by the empirically determined ratio of the mean molecular weight of cholesterol esters to the molecular weight of cholesterol (1.677, mean ratio for 44 healthy adults, R. Akins *et al.* unpublished data). This formula is very similar to one derived by Cheek and Wease (1969), except that free cholesterol is actually measured rather than assuming it to be a constant proportion (27%) of total cholesterol.

[2] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

An alternative formula for estimating total serum lipids that uses only total cholesterol and triglyceride values was also applied:

$$TL = 2.27\,TC + TG + 0.623 \qquad (2)$$

This formula incorporates predicted phospholipid content derived from a regression of phospholipids on serum total cholesterol in 81 samples from a previous study (Patterson *et al.* 1988). It further assumes that free cholesterol is 27% of total cholesterol (Cheek and Wease 1969). The phospholipid regression equation

$$PL = 0.766\,TC + 0.623 \qquad (3)$$

was very similar to that previously reported by Cheek and Wease (1969).

Because the 80 serum concentration values for each chlorinated hydrocarbon (CH) represent 4 determinations made on each of 20 subjects, a randomized block design analysis of variance (Sokal and Rohlf 1969) was used to block out differences among subjects before determining the significance of the differences in CH concentration at the 4 sampling times. A separate analysis was done for each CH. The dependent variable was CH concentration, the blocking variable was person, and the treatment variable was sampling time. If significant differences were found by the analysis of variance F-test for sample times, Tukey's Honestly Significant Difference (HSD) test (Sokal and Rohlf 1969) was used to locate the pairwise differences in concentrations between sample times. The statistical analysis was performed on the entire data set and also on males and females separately to determine if there were any gender-specific differences in the effect of lipid correction. All statistical analyses were carried out using the SAS[2] statistical software package (SAS Institute 1985). A significance level of 0.05 was used for all tests.

Results

On a serum weight basis, both nonfasting serum samples had significantly higher ($p < 0.05$) mean concentrations of PCBs, HCB, and *p,p'*-DDE than did either of the fasting samples, with the exception of one fasting sample for *p,p'*-DDE (Table 1). There were no significant differences ($p > 0.05$) between the two nonfasting samples or between the two fasting samples. Overall, the nonfasting samples had mean concentrations that were higher than the corresponding fasting sample means by 29% for PCBs (4.81 vs 3.74 ng/g serum), 22% for HCB (0.163 vs 0.134 ng/g serum), and 26% for *p,p'*-DDE (6.74 vs 5.37 ng/g serum).

Total serum lipids (Equation 1) for nonfasting samples averaged 7.05 g/L, a 20% increase over the mean of 5.86 g/L in fasting samples (Table 2). Triglycerides accounted for 87% of this increase, rising 122% from 0.85 g/L in fasting samples to 1.88 g/L in nonfasting samples. Total cholesterol decreased 2% from 1.96 g/L to 1.91 g/L. Free cholesterol increased 5% from 0.53 g/L to 0.55 g/L, and

Table 1. Mean chlorinated hydrocarbon concentrations for 20 adults at 4 sampling times on a serum weight, lipid weight, and triglyceride weight basis

	Nonfasting		Fasting		
Chlorinated Hydrocarbon	Pre-Lunch	Mid-Afternoon	1st Morning	2nd Morning	Pooled[a] S.D.
PCBs[c] (ng/g serum wt)	4.88	4.75[b]	3.80	3.68	0.68
HCB[d] (ng/g serum wt)	0.163	0.164	0.138	0.130	0.021
p,p'-DDE[e] (ng/g serum wt)	6.77	6.70	5.68	5.06	1.33
PCBs (μg/g lipid, Eq. 1)	0.666	0.668	0.622	0.630	0.066
HCB (μg/g lipid, Eq. 1)	0.0227	0.0230	0.0229	0.0224	0.0021
p,p'-DDE (μg/g lipid, Eq. 1)	0.952	0.953	0.943	0.892	0.116
PCBs (μg/g lipid, Eq. 2)	0.681	0.693	0.618	0.622	0.069
HCB (μg/g lipid, Eq. 2)	0.0232	0.0239	0.0227	0.0221	0.0022
p,p'-DDE (μg/g lipid, Eq. 2)	0.984	0.970	0.935	0.875	0.126
PCBs (μg/g triglyceride)	2.56	3.13	4.36	5.21	0.96
HCB (μg/g triglyceride)	0.0898	0.111	0.166	0.189	0.033
p,p'-DDE (μg/g triglyceride)	3.84	4.75	6.82	7.78	1.89

[a] Pooled standard deviation is the root mean square error from the randomized block design ANOVA. This is the S.D. term used for the Tukey's HSD tests of significant differences among sampling times

[b] Means sharing a common underline are not significantly different from each other ($p < 0.05$, F-test on chlorinated hydrocarbon concentrations, followed by Tukey's HSD test to locate pairwise differences if present)

[c] Polychlorinated biphenyls

[d] Hexachlorobenzene

[e] *p,p'*-dichlorodiphenyldichloroethylene

Table 2. Mean concentrations of serum lipids in 2 nonfasting samples and 2 fasting samples for 10 male and 10 female adults

Lipid Component	Mean (SD) – Nonfasting Samples (g/L)	Mean (SD) – Fasting Samples (g/L)	Diff.[a] (NF-F)/F
Triglycerides	1.88 (0.86)	0.85 (0.38)	122
Total Cholesterol	1.91 (0.34)	1.96 (0.34)	−2
Free Cholesterol	0.55 (0.11)	0.53 (0.09)	5
Phospholipids	2.34 (0.29)	2.08 (0.24)	12
Total Serum Lipids (Eq. 1)	7.05 (1.34)	5.86 (0.95)	20
Total Serum Lipids (Eq. 2)	6.84 (1.32)	5.92 (0.98)	16

[a] % difference between means for nonfasting and fasting samples, expressed as a percentage of the mean for fasting samples

phospholipids increased 12% from 2.08 g/L to 2.34 g/L. Total serum lipids as measured by Equation 2 rose 16% from 5.92 g/L in fasting samples to 6.84 g/L in nonfasting samples.

When CH concentrations were corrected for total serum lipids by Equation 1 and expressed on a lipid weight basis, there were no significant differences ($p > 0.05$) among measurements at any of the sample times for any of the three analytes (Table 1). Differences between nonfasting and fasting means (nonfasting minus fasting) were reduced to 7% for PCBs (0.667 vs 0.626 μg/g lipid), 1% for HCB (0.229 vs 0.227 μg/g lipid), and 4% for *p,p'*-DDE (0.953 vs 0.918 μg/g lipid).

Repeating the analyses, while stratifying by gender, gave similar results for both sexes: nonfasting samples had significantly higher mean CH concentrations than did fasting samples on a serum weight basis, but there were no significant differences on a lipid weight basis. Since the outcome was identical, stratification by gender is not necessary and these results are not shown. Males had 19% to 34% higher CH concentrations on a serum weight basis, but similar to only 15% higher CH concentrations on a lipid weight basis than did females (Table 3).

Lipid correction by Equation 2, which uses only total cholesterol and triglyceride values, was not as successful in eliminating differences between nonfasting and fasting samples. The nonfasting samples

Table 3. Comparison of chlorinated hydrocarbon concentrations between males and females. Mean concentrations over the 4 sampling times were used for each person (10 males and 10 females)

	Serum weight (ng/g serum)						
Chlorinated Hydrocarbon	Males			Females			% Diff.[a] (M-F)/F
	Mean	S.D.	n	Mean	S.D.	n	
PCBs[b]	4.81	1.84	10	3.74	1.03	10	29
HCB[c]	0.170	0.058	10	0.127	0.009	10	34
p,p'-DDE[d]	6.57	4.62	10	5.53	2.79	10	19
	Lipid weight (μg/g lipid, Eq. 1)						
Chlorinated Hydrocarbon	Males			Females			% Diff. (M-F)/F
	Mean	S.D.	n	Mean	S.D.	n	
PCBs	0.683	0.254	10	0.610	0.257	10	12
HCB	0.0243	0.0065	10	0.0212	0.0030	10	15
p,p'-DDE	0.919	0.578	10	0.951	0.555	10	−3

[a] % difference between means for males and females, expressed as a percentage of the mean for females
[b] Polychlorinated biphenyls
[c] Hexachlorobenzene
[d] *p,p'*-dichlorodiphenyldichloroethylene

had significantly higher ($p < 0.05$) CH concentrations on a lipid weight basis than did both fasting samples for PCBs and one fasting sample for *p,p'*-DDE (Table 1). Nearly significant differences ($0.05 < p < 0.10$) remained for HCB. Overall, nonfasting concentrations on a lipid weight basis were elevated above fasting levels by 11% for PCBs (0.687 vs 0.620 μg/g lipid), 5% for HCB (0.0236 vs 0.0224 μg/g lipid), and 8% for *p,p'*-DDE (0.977 vs 0.905 μg/g lipid).

Expressing chlorinated hydrocarbon levels on a triglyceride weight basis (correction by triglycerides alone) resulted in considerable overcorrection. Means for fasting samples were significantly higher than those for nonfasting samples (Table 1): 68% higher for PCBs (4.79 vs 2.85 μg/g triglyceride, 77% higher for HCB (0.177 vs 0.100 μg/g triglyceride), and 70% higher for *p,p'*-DDE (7.30 vs 4.30 μg/g triglyceride).

Discussion

Lipophilic CHs are not in solution in the aqueous portion of blood, but form nonspecific bonds with hydrophobic sites on blood proteins, especially lipoproteins, and with similar sites on the cellular component of blood (Matthews 1980; Matthews *et al.* 1984; Birnbaum 1985). This partitioning takes place quickly and is determined by the lipid content of the various blood components and the lipid solubility of the CH compound (Maliwal and Guthrie 1981, 1982; Matthews *et al.* 1984). Animal studies have shown that PCBs are quickly removed from the blood into muscle and liver, which have high perfusion rates and some affinity for the compounds (Matthews and Anderson 1975). Redistribution to adipose tissue, with a much higher affinity due to its high lipid content, takes place more slowly, and eventually equilibrium is reached among all tissues (Matthews and Anderson 1975). As with blood components, equilibrium levels among tissues are determined by their lipid content and the lipid solubility of the CH (Lutz *et al.* 1977).

Because the distribution of CHs among tissues is a dynamic equilibrium, a change in the lipid content of one tissue would alter the equilibrium. These changes would likely occur rapidly for blood, liver, and muscle and more slowly for adipose tissue. Consequently, postprandial increases in serum lipids might be expected to proportionately affect serum CH levels. We observed exactly that effect. Increases of 20% in total serum lipid levels led to similar size increases in serum PCB, HCB, and *p,p'*-DDE concentrations. When CH levels were expressed on a lipid weight basis (μg/g lipid), there were no longer significant differences between fasting and nonfasting samples. Brown and Lawton (1984) argue for expression of lipophilic xenobiotic chemical concentrations on a lipid weight basis as a direct measure of the chemical activity to which pharmacological responses are proportional.

Chlorinated hydrocarbon levels on a serum weight basis have often been observed to be higher

in males than in females (*e.g.*, this study, Kreiss *et al.* 1981a, 1981b). Adult males, ages 25–50, however, also tend to have somewhat higher blood cholesterol and triglyceride levels than females of the same age (National Heart, Lung, and Blood Institute 1980). In the present study, males had 15% higher total serum lipid levels than did females. Thus, gender-based differences in serum weight CH concentrations may be due to differences in body burden and/or differences in serum lipid levels. When CH levels were expressed on a lipid weight basis, the magnitude of the differences in chlorinated hydrocarbon levels between males and females decreased (Table 3). Thus, gender-based differences in serum concentrations of lipophilic compounds may be overestimated by the use of uncorrected serum weight measures.

Correction for total serum lipids based on total cholesterol and triglyceride levels alone (Equation 2) made fasting and nonfasting serum CH levels more comparable but still significantly ($p < 0.05$) or nearly significantly ($p < 0.10$) different (Table 1). This short formula does not account for the small postprandial increases observed in free cholesterol (5%) and phospholipids (12%) and consequently underestimates the change in total serum lipids as determined by Equation 1.

Triglycerides accounted for the greatest portion (87%) of the 1.19 g/L increase in total serum lipids between fasting and postprandial states. Triglycerides exhibited a 122% increase, while total serum lipids rose only 20%. Serum CH levels increased in approximately the same proportion (22% to 29%) as did total serum lipids. While most of the increase in serum lipid content may be due to a rise in triglycerides, correction for triglycerides overcompensates for increased serum CH concentrations. CHs seem to be repartitioning in response to overall serum lipid changes rather than to changes in any one lipid component.

In summary, significant differences in serum concentrations of lipophilic CH compounds, such as PCBs, HCB, and *p,p'*-DDE, may be due to serum lipid variations associated with fasting and postprandial states. These differences affect the comparability of measurements made on samples collected under different specimen collection regimens. Expressing CHs on a lipid weight basis, however, produces comparable results regardless of whether the subject fasted before sample collection. This finding is important to properly design and interpret studies involving serum levels of CHs. Correction for total serum lipids may still be important, even if fasting samples are to be obtained, because there is often uncertainty as to whether participants were truly fasting before blood collection. A simple expression of serum lipids, based only on total cholesterol and triglycerides, was only partly successful, however, and correction by triglycerides alone overcompensated for serum CH increases. A rather complete characterization of the total serum lipid components was required to adequately correct the data.

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