Increase in Plasma Pollutant Levels in Response to Weight Loss Is Associated With the Reduction of Fasting Insulin Levels in Men But Not in Women

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Environmental pollutants can act as endocrine modulators. In this study, we examined whether weight loss-induced changes in plasma organochlorine compounds (OC) were associated with those in plasma insulin levels. Fasting insulin and the area under the curve (AUC) of insulin after a 75-g oral glucose load, plasma levels of 1 commercial polychlorinated biphenyl (PCB) mixture (Aroclor 1260), 1 PCB congener (PCB 153), and 3 pesticides (2,2′-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p′*-DDE), beta-hexachlorocyclohexane (β-HCH), and hexachlorobenzene (HCB)) were measured before and after a 15-week weight loss program induced by a caloric restriction in a sample of obese men and women. Both genders showed a similar reduction in body weight (≈ 11 kg) in response to treatment, although men lost significantly more fat mass than women (mean ± SD 9.4 ± 4.1 *v* 5.9 ± 5 kg, respectively, $P < .05$). Fasting insulin and AUC of insulin significantly decreased in men and women after the treatment. In response to weight loss, a significant increase in OC was observed in both genders, and this effect was more pronounced in men. The greater the increase in plasma OC levels, the greater the reduction in fasting insulin was in response to weight loss in men ($-.49 < r < -.59$, $P < .05$), but not in women ($-.22 < r < .01$, not significant [NS]). In both genders, no relationship was observed between changes in plasma OC levels and changes in AUC of insulin ($-.41 < r < -.08$, NS). In men, relationships between changes in plasma HCB, Aroclor 1260, and PCB-153 concentrations and those in fasting insulin levels in response to weight loss remained significantly correlated after correction for fat mass loss ($-.46 <$ partial $r < -.51$, *P* values ranging from .05 to .07). These results suggest that weight loss-induced increase in plasma pollutant levels tends to be independently associated with the reduction of fasting insulin levels in men, but not in women. Further studies are needed to verify whether these findings are causally related.

THE INDUSTRIALIZATION ERA for most western countries has been characterized by the production of organochlorine compounds (OC). These man-made chemicals include agricultural and industrial compounds, as well as by-products of industrial processes involving chlorine chemistry and combustion of fuels. Due to their persistence and their lipophilicity, these compounds preferentially bioaccumulate in higher trophic levels of the food chain.[1,2] Consequently, OC are still found in virtually every person on the planet and might play adverse effects in humans.[1,3] One potential endocrine disorder, which has been associated with high plasma levels of chemicals, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a dioxin contained in the herbicide mixture of Agent Orange, is diabetes. Indeed, Henriksen et al[4] showed in a prospective study that individuals with high-serum TCDD levels displayed a greater prevalence of diabetes and a shorter time to onset of diabetes, as compared with those with low-blood TCDD levels. Concordant with this observation, plasma insulin concentrations, at fasting and following a 75-g glucose load, have recently been shown to be significantly higher in subjects with higher TCDD levels.[5] Studies of polychlorinated biphenyl (PCB)-exposed workers show no suggestion of an increased level of glucose,[6,7] whereas Longnecker et al[8] recently reported that the mean serum levels of PCB among pregnant subjects with diabetes was significantly higher than in control subjects.

To our knowledge, no study has yet considered the potential impact of a significant plasma level increase of OC on insulin levels in the same individual. The major reason for this is that, ethically, it is unacceptable to study the impact of contaminants by purposely exposing individuals to suspected pollutants. Nevertheless, the increase in plasma OC levels that we recently observed following weight loss in obese individuals[9] represents a valuable model to verify whether insulin levels might be influenced by pollutants. Therefore, the aim of this study was to examine whether the weight loss-induced increase in plasma OC levels of 17 men and 20 women was associated with changes in fasting insulin and in insulin response measured during a glucose load.

MATERIALS AND METHODS

Subjects

A total of 17 men and 20 women, all Caucasians, were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication, which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc), were excluded from the study. All participants were sedentary (ie, fewer than 2 exercise sessions of 30 minutes/week), nonsmokers, and moderate alcohol consumers (ie, fewer than 140 g/week). None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last 6 months prior to the study.

All subjects participated in a 15-week nonmacronutrient-specific energy restriction of -2,930 kJ/d combined with drug therapy (fenfluramine 60 mg/d) or placebo, as previously described.[10] This nonmacronutrient-specific energy restricted diet was fixed, in part, with a

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resting metabolic rate (RMR) measurement to which an activity factor of 1.4[11] was multiplied to estimate daily energy expenditure (DEE) of subjects.

Noteworthy is the fact that following the suspension of fenfluramine and dexfenfluramine further to a potential association with disturbances in cardiac valvular function,[12,13] all subjects (including placebo) underwent an echocardiogram. Following this measure, a detailed analysis of cardiac valvular function was performed by cardiologists who detected no abnormalities in response to the use of fenfluramine under these conditions.

Total Body Fatness and Regional Fat Distribution

Body weight was taken with a standard beam scale. Body density was determined by the underwater weighing technique from which percent body fat was derived with the Siri formula.[14] Pulmonary residual volume was measured using the helium dilution method.[15] Fat mass and fat-free mass were derived from the percentage of body fat and total body weight. Computed tomography (CT) was performed with a Siemens Somatom DRH scanner (Erlangen, Germany) according to the methodology previously described by Sjöström et al.[16] Briefly, subjects were examined in the supine position with both arms stretched above the head. The CT scan was performed at the abdominal (between L4 and L5 vertebrae) level, using a scout radiograph to establish the position of the scan to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU).[17] The abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was determined by subtracting the visceral AT area from the total abdominal AT area.

Oral Glucose Tolerance Test

A 75-g oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast. Blood samples were collected in tubes containing EDTA and trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubial vein at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. Plasma insulin concentrations were determined by radioimmunoassay with polyethylene glycol separation[18] (Linco Research, St Louis, MO), and plasma glucose levels were determined using the glucose oxidase assay[19] (Sigma, St Louis, MO). The total insulin and glucose areas under the curve (AUC) during OGTT were calculated with the trapezoid method.

Chemical Analysis

Based on our previous observations[9] and to simplify the statistical analyses, only the most abundant organochlorines found in plasma were considered. Briefly, 1 PCB congener (International Union of Pure and Applied Chemistry, no. 153), 1 commercial PCB mixture formerly used in electrical transformers, Aroclor 1260, and 3 chlorinated pesticides (2,2'-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), beta-hexachlorocyclohexane [β-HCH], and hexachlorobenzene [HCB]) were determined in plasma samples. Blood samples were taken before and following weight loss. Samples were first spiked with the internal standard (PCB congener no. 198), homogenized in hexane:acetone (2:1, vol/vol), and the resulting organic phase washed with water to remove the bulk of the acetone. An aliquot of the hexane extract was used for lipid determination by gravimetry, and the rest of the extract was defatted with concentrated sulfuric acid. The defatted hexane was successively washed with water and aqueous potassium hydroxide prior to filtration through anhydrous sodium sulfate. The filtrate was then concentrated and cleaned up by chromatography on an acidic silica gel column and a deacrivated (0.5%) Florisil column. Organochlorines were eluted from the columns using methylene chloride: hexane (25:75, vol/vol) and analyzed on a HP-5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with dual capillary columns (Ultra-1 and Ultra-2) and dual ^{63}Ni electron detectors. Detection limits varied from 0.02 to 0.3 μg/L. All plasma concentrations have been transformed on a lipid weight basis (μg/kg), because organochlorines are lipophilic substances that distribute in body lipids.

Statistical Analyses

Pretreatment gender differences were tested for significance with the Student's *t* test. Multivariate analysis of variance (MANOVA) for repeated measures were performed on all variables to assess the effects of treatment and gender over time. Neither treatment effect nor sex by treatment interaction was observed. As no treatment and time interaction was noted for all variables investigated, placebo and drug-treated individuals were pooled. Identification of a significant sex by time interaction led to further analysis of a simple main effect for sex, and post hoc analysis was tested with a paired *t* test. Univariate associations between variables were quantified using Pearson's product moment correlation coefficients. Finally, partial correlation was performed to assess the relationship between 2 variables with the effect of a third variable eliminated. The change in variables was determined as the difference between post- minus preweight loss values. Results of the present study were similar when changes were expressed in percentages. Statistical significance was defined as *P* less than .05. All analyses were performed using JMP software from SAS Institute Inc (Cary, NC) on Macintosh computers.

RESULTS

The physical and metabolic characteristics before and after weight loss of obese men and women are presented in Table 1. Pretreatment body weight and visceral AT levels were higher in men, whereas percent body fat, fat mass, and subcutaneous fat accumulation were higher in women ($P < .05$). Both genders showed a similar reduction of body weight and subcutaneous abdominal fat accumulation in response to treatment. However, significant sex by time interactions were found for percent body fat, fat mass, and visceral fat accumulation, showing that men lost significantly more fat mass, percent body fat, and visceral AT than women. Fasting plasma insulin levels and the AUC of insulin significantly decreased in men and women in response to weight loss ($P < .05$). Weight loss did not significantly change fasting glucose and the AUC of glucose in both genders (data not shown). In addition, before weight loss, fat mass and fasting plasma insulin were positively correlated in men ($r = .32$, not significant [NS]) and women ($r = .48$, $P < .05$) (data not shown). In response to weight loss, the greater the decrease in fat mass, the greater the decrease in fasting insulin was in women ($r = .45$, $P < .05$), although this relationship failed to reach statistical significance in men ($r = .30$, NS) (data not shown).

Plasma organochlorine levels were comparable in men and women before weight loss (Table 2). Except for β-HCH, which increased in a similar way in response to weight loss in both genders, sex by time interactions were found for all organochlorines investigated in response to weight loss. Plasma levels of *p,p'*-DDE, Aroclor 1260, and PCB 153 were significantly increased in response to caloric restriction in both genders (*P* values ranging from .001 to .05), this effect being more pronounced in men. A significant increase in plasma levels of HCB was also observed in men ($P < .05$), whereas plasma concen-

Table 1. Physical and Metabolic Characteristics of Men and Women Before and After Weight Loss

	Men (n = 17)		Women (n = 20)				
	Before	After	Before	After	Sex	Time	Sex/Time
Weight (kg)	105 ± 10	94 ± 9	91 ± 14	82 ± 13	.01	.0001	NS
BMI (kg/m^2)	34 ± 3	30 ± 3	36 ± 4	33 ± 5	NS	.0001	NS
Body fat (%)	38 ± 5	30 ± 5*	48 ± 4	46 ± 5†	.0001	.0001	.0001
Fat mass (kg)	39 ± 7	29 ± 5*	45 ± 9	39 ± 10*	.01	.0001	.05
Adipose tissue areas measured by CT (cm^2)							
Abdomen (L4-L5)							
Subcutaneous	402 ± 67	326 ± 68	551 ± 141	489 ± 150	.001	.0001	NS
Visceral	202 ± 60	132 ± 50*	149 ± 45	118 ± 37*	.05	.0001	.01
Insulin (pmol/L)	123 ± 52	93 ± 50	96 ± 68	86 ± 67	NS	.001	NS
Insulin area	116 ± 45	96 ± 36	102 ± 42	91 ± 38	NS	.01	NS

NOTE. Values are means ± SD. Insulin area represents integrated plasma concentrations measured for 3 hours after an oral glucose load (75 g/OGTT) and is expressed in (pmol/L/min) × 10^{-3}.

Abbreviations: BMI, body mass index; CT, computed tomography; NS, not significant.

Statistical within group difference at *$P < .001$ and †$P < .01$.

trations of this pollutant remained unchanged following weight reduction in women.

Before weight loss, no significant correlation was observed between fasting plasma insulin and plasma organochlorine levels in both genders ($-.02 < r < .29$; NS) (data not shown). Moreover, the AUC of insulin was not significantly correlated to plasma OC levels in men, whereas significant positive associations were observed in women between the AUC of insulin and most of the pollutants investigated (HCB, β-HCH, Aroclor 1260, and PCB 153) ($.49 < r < .67$, P values ranging from .01 to .05) (data not shown). In response to weight loss, we found that the greater the increase in plasma OC levels, the greater the decrease in fasting insulin concentrations was in men ($-.49 < r < -.59$, $P < .05$), but not in women ($-.22 < r < .01$, NS) (Table 3). To verify whether the previous relationships in men were independent of changes in fat mass, partial correlations have been performed. Negative partial relationships were observed between variations in HCB, Aroclor 1260, and PCB 153 levels and those in fasting insulin levels ($-.46 <$ partial $r < -.51$, P values ranging from .05 to .07) (Fig 1). Moreover, changes in HCB, Aroclor 1260, and PCB 153 levels and those in fasting insulin levels remained significantly correlated after correction for changes in visceral AT accumulation ($-.51 <$ partial $r < -.53$, $P < .05$) (data not shown). In both genders, no significant relationship was observed between changes in plasma OC levels and those in insulin response after a glucose load in response to weight loss ($-.41 < r < -.08$, NS) (data not shown). Moreover, changes in OC levels were not significantly correlated to those in fasting glucose or glucose response to OGTT in men and in women ($-.25 < r < .41$, NS) (data not shown).

DISCUSSION

The present study showed that changes in some plasma OC concentrations were associated with changes in fasting insulin levels in men even after controlling for fat mass or visceral AT loss. On the other hand, no relationship was observed between changes in plasma OC and insulin levels in response to weight loss in women.

Evidence in man indicates that environmental pollutants can act as endocrine modulators and then jeopardize human health.[1,3] Among the concerns is the impact of pollutants on plasma glucose homeostasis. Indeed, serum concentrations of TCDD have previously been found to be positively associated with glucose concentrations in humans.[4] Moreover, a greater mortality risk for diabetes,[20] as well as a higher prevalence of diabetes,[4,21] has been reported in individuals with high- versus low-blood TCDD levels. These observations are concordant with our results in women before weight loss showing positive associations between the AUC of insulin and the plasma OC levels. On the other hand, no significant relationship was found

Table 2. Plasma Organochlorine Levels (μg/kg) of Men and Women Before and After Weight Loss

	Men (n = 17)		Women (n = 20)				
	Before	After	Before	After	Sex	Time	Sex/Time
p,p′-DDE	423 ± 174	527 ± 229*	366 ± 237	405 ± 267†	NS	.0001	.05
β-HCH	22 ± 19	28 ± 25	16 ± 7	18 ± 9	NS	.01	NS
HCB	21 ± 6	26 ± 6†	22 ± 11	24 ± 10	NS	.0001	.05
Aroclor 1260	515 ± 220	635 ± 236*	421 ± 200	460 ± 185†	NS	.0001	.01
PCB 153	57 ± 22	71 ± 24*	47 ± 22	52 ± 21‡	.05	.0001	.01

NOTE. Values are means ± SD.

Abbreviation: NS, not significant.

Statistical within group difference at *$P < .001$, †$P < .05$, and ‡$P < .01$.

before weight loss between OC levels and either the fasting insulin levels or the area under the plasma insulin curve during an OGTT in men. The gender dimorphism regarding the influence of OC on the AUC of insulin appears difficult to explain, because men and women presented similar plasma OC levels before weight loss. Further studies remain to be performed to elucidate this observation.

The fact that changes in fasting insulin or insulin area and those in pollutants in response to weight loss were not significantly correlated in women suggests that the weight loss-induced OC levels reported in the present study unalters their glucose homeostasis. This absence of a relationship may be due to the smaller increase in plasma OC levels in response to weight loss observed in women than in men. Accordingly, this assumption raises the question as to whether a substantial weight reduction presents a greater potential to trigger harmful effects on glucose homeostasis than a moderate one? As body weight loss is associated with OC increase[9] and as the risk of hyperinsulinemia is observed over a certain level of pollutants,[4,5,21] it is likely that a certain weight loss-induced increase in the plasma OC level threshold is required for altering glucose and/or insulin metabolism.

We found that the greater the increase in some OC levels, the greater the decrease in fasting insulin concentrations was following weight loss in men, regardless of the fat loss variation. This finding suggests that the increase in some OC levels following a weight reduction in men is associated with at least one of the signal transduction pathways between glucose and insulin secretion independently of changes in fat mass. Our results appear to run counter to what would have been expected based on studies, which reported that high levels of TCDD are related to high serum glucose or insulin concentrations.[4,5] However, one should keep in mind that one of the earliest event occurring after poisoning animals with TCDD is a decrease of circulating insulin.[22] One potential mechanism explaining this observation is that TCDD induces an increase in protein tyrosine kinase activities in the pancreas, which alters insulin secretion.[23] Although TCDD takes part of the ubiquitous and persistent polychlorinated aromatic hydrocarbons as the OC investigated in the present study, whether or not the mechanism by which plasma OC levels can modulate insulin secretion in men is similar to that of TCDD in animals is, as yet, unknown.

Table 3. Relationships Between Changes (Δ) in Plasma Organochlorine Levels and Those in Fasting Insulin in Obese Men and Women in Response to Weight Loss

	Δ Fasting Insulin	
	Men	Women
Δ *p,p'*-DDE	−.49*	−.17
Δ β-HCH	−.53*	.01
Δ HCB	−.57*	−.27
Δ Aroclor 1260	−.59†	−.15
Δ PCB 153	−.57*	−.22

Statistical significance at $*P < .05$, $†P < .01$.

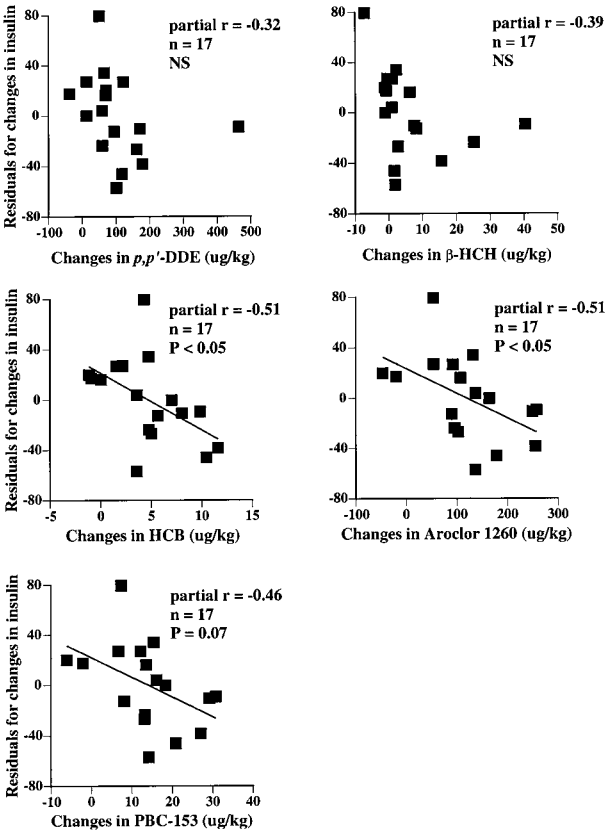

Fig 1. Relationships between changes in plasma organochlorine and those in fasting insulin corrected for changes in fat mass in obese men in response to weight loss.

In summary, plasma organochlorine levels significantly increased in response to a moderate weight loss in obese individuals. In men, the increase in some plasma OC was associated with the reduction in fasting insulin even after controlling for fat mass or visceral AT loss. On the other hand, no significant relationship between changes in plasma OC levels and fasting insulin concentrations was observed in women. More studies are needed to further clarify how the toxic substances found in plasma may be involved in the control of insulin secretion in humans in response to weight loss and to what extent this could differ between men and women.

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