Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity

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Obesity is commonly associated with an increase in skeletal muscle triglyceride deposition (9, 14), a feature associated with insulin resistance (12, 18, 24). Recently, Kelley et al. (11) outlined the mechanism that could account for the increase in skeletal muscle lipid deposition occurring in obesity by reporting reduced skeletal muscle capacity for fat oxidation rather than increased fatty acid uptake in obese individuals. In this previous study, it was also reported that the fasting pattern of fatty acid oxidation by muscle did not improve after weight loss. This observation is supported by recent data showing alterations in mitochondrial skeletal muscle markers of fatty acid metabolism after dietary restriction (25). Such markers include β-hydroxyacyl-CoA dehydrogenase (HADH), citrate synthase (CS), and cytochrome c oxidase (COX) activities. This finding appears consistent with previous data having reported persistent alteration of fatty acid metabolism after weight loss (1, 27), an observation of clinical importance because a decreased reliance on lipid oxidation has already been shown to be a risk factor for weight gain (29).

We recently reported that an adequate diet aiming at weight loss induced a significant increase in plasma organochlorine (OC) levels (3). OC are man-made chemicals, which include agricultural and industrial compounds as well as by-products of industrial processes involving chlorine chemistry and combustion of fuels. Because of their persistence and their lipophilicity, these compounds preferentially bioaccumulate in higher trophic levels of the food chain (15, 22). Consequently, OC are found in virtually every person on the planet and may have adverse effects on human health (8, 22). In this regard, OC have previously been shown to induce inhibition in enzyme activities of the mitochondrial electron transport chain (19). More recently, Narasimhan et al. (17) also reported that polychlorinated biphenyls (PCBs) have multiple inhibitory sites on the mouse liver mitochondrial electron transport system. So far, little is known regarding the effect of OC on mitochondrion oxidative capacity of human skeletal muscle. On the basis of the previous observations, it is likely that weight loss-induced increase in plasma pollutant levels is likely to be associated with reduced skeletal muscle oxidative metabolism in men but not in women.


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Table 1. Physical characteristics of men and women before and after weight loss

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 17)</th>
<th>Women (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>105 ± 10</td>
<td>94 ± 9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>34 ± 3</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Abdomen circumference, cm</td>
<td>116 ± 6</td>
<td>107 ± 7ₚ</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>38 ± 5</td>
<td>30 ± 5*</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>39 ± 7</td>
<td>29 ± 5*</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>66 ± 7</td>
<td>65 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Statistical within-group difference at *P < 0.05, †P < 0.01, and ‡P < 0.001. NS, not significant.

MATERIAL AND METHODS

Subjects. Seventeen men and twenty 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, and endocrine disorders, or those on medication that could have influenced triglyceride metabolism (such as β-blockers and antihypertensive drugs), were excluded from the study. All participants were sedentary (i.e., fewer than two exercise sessions of 30 min/wk), nonsmokers, and moderate alcohol consumers (i.e., <140 g/wk). None was working in an environment where the risk of exposure to OC was high. 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 mo before the study.

All subjects participated in a 15-wk nonmacronutrient-specific energy restriction of −2,930 kJ/day combined with drug therapy (fenfluramine 60 mg/day) or placebo, as previously described (4). This nonmacronutrient-specific energy-restricted diet was determined by estimating daily energy needs with a resting metabolic rate (RMR) measurement that was multiplied by an activity factor of 1.4 (28). The prescribed energy intake was determined by subtracting the energy restriction from daily energy needs.

Anthropometric evaluation. Body weight was taken with a standard beam scale. Abdomen circumference was taken according to Lohman et al. (13). Body density was determined by the underwater weighing technique, from which percent body fat was derived with the Siri formula (26). Pulmonary residual volume was measured using the helium dilution method (16). Fat mass and fat-free mass were derived from the percentage of body fat and total body weight.

Skeletal muscle biopsies. Muscle samples were obtained before and 4–6 wk after the weight loss intervention. Biopsies were taken from 15 cm above the patella and −2 cm away from the fascia by use of the percutaneous needle biopsy technique previously described by Evans et al. (5). Muscle samples were frozen in liquid nitrogen and kept at −80°C until they were assayed for enzyme activities.

Skeletal muscle enzyme activities. Small pieces of the muscle sample (~10 mg) were homogenized in a glass-glass Duall homogenizer with 39 vol of ice-cold extracting medium (0.1 M Na-K-phosphate, 2 mM EDTA, pH 7.2). Homogenate was transferred into 1.5-ml polypropylene tubes to be magnetically stirred on ice for 15 min and finally sonicated six times for 5 s at 20 W on ice with pauses of 85 s between pulses. The resulting homogenate was used for determination of activity (Vmax) of phosphofructokinase (PFK; EC 2.7.1.11), HADH (EC 1.1.1.35), CS (EC 4.1.3.7), and COX (EC 1.9.3.1), as previously described (7).

Chemical analysis. On the basis of our previous observations (3) and to simplify the statistical analyses, only the most abundant OCs found in plasma were considered. Briefly, one PCB congener (International Union of Pure and Applied Chemistry no. 153), one commercial PCB mixture formerly used in electrical transformers (Aroclor 1260), and three chlorinated pesticides (2,2'-bis(4-chlorophenyl)-1,1-di-chloroethene), (p,p'-DDE), β-hexachlorocyclohexane (β-HCH), and hexachlorobenzene (HCB) were determined in plasma samples at the Quebec Toxicology Center. Blood samples were taken before and after weight loss and were centrifuged to extract plasma (2 ml), which was cleaned up by chromatography on an acidic silica gel column and a deactivated (0.5%) Florisil column. Plasma samples were eluted from the columns using methylene chloride-hexane (25:75, vol/vol) and analyzed on an HP-5890 gas chromatograph equipped with two capillary columns (Ultra-1 and Ultra-2) and dual 63Ni electron detectors. Peaks were identified by their relative retention times obtained on the two columns using a computer program developed by the Quebec Toxicology Center. Depending on the compounds, detection limits varied from 0.02 to 0.3 µg/l. Plasma lipid concentration was also determined by enzymatic methods on a Technicon automatic analyzer (RA-500) with the following test packs: Randox for total cholesterol and triglycerides, BMC for free cholesterol,

Table 2. Skeletal muscle enzyme activities of men and women before and after weight loss

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 17)</th>
<th>Women (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>PFK</td>
<td>63 ± 9</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>HADH</td>
<td>15 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>CS</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>COX</td>
<td>7 ± 2</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed in µmol·min⁻¹·g⁻¹. PFK, phosphofructokinase; HADH, β-hydroxyacyl-CoA dehydrogenase; CS, citrate synthase; COX, cytochrome c oxidase. Statistical within-group difference at *P < 0.05, †P < 0.01, and ‡P < 0.001.
and Wako for phospholipids. Plasma total lipids were calculated with the equation recommended by Patterson et al. (20)

\[
\text{total lipids} = 1.677(\text{TC} - \text{FC}) + \text{FC} + \text{TG} + \text{PL}
\]

All plasma concentrations have been transformed on a lipid weight basis (μg/kg), because OCs are lipophilic substances that distribute in body lipids.

**Statistical analyses.** Pretreatment sex differences were tested for significance with the Student’s t-test. Multivariate analysis of variance (MANOVA) for repeated measures was performed on all variables to assess the effects of treatment and gender over time. As no treatment and time interaction was noted for all variables investigated, data from placebo- and drug-treated individuals were pooled. Identification of a significant sex × 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 two 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 percentage. Statistical significance was defined as \(P < 0.05\). All analyses were performed using JMP software from SAS Institute (Cary, NC) on Macintosh computers.

**RESULTS**

The physical characteristics of obese men and women before and after weight loss are presented in Table 1. Pretreatment body weight and fat-free mass levels were higher in men, whereas percent body fat and fat mass were higher in women \((P < 0.01)\). Men and women showed a similar reduction of body weight and fat-free mass in response to treatment. However, significant sex × time interactions were found for abdomen circumference, percent body fat, and fat mass, revealing that men lost significantly more of these parameters than women.
Skeletal muscle enzyme activities of men and women before and after weight loss are shown in Table 2. PFK levels were greater in men than in women before and after weight loss \( (P < 0.001) \). In both sexes, mean PFK, HADH, CS, as well as COX activities, remained unchanged in response to treatment.

Plasma OC levels were comparable in men and women before weight loss, as shown in Fig. 1. Except for \( \beta \)-HCH, which increased in a similar way in response to weight loss in both sexes, sex \( \times \) time interactions were found for all OC 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 sexes \( (P \) values ranging from 0.001 to 0.05), this effect being more pronounced in men. A significant increase in plasma levels of HCB was also observed in men \( (P < 0.05) \), whereas plasma concentrations of this pollutant remained unchanged after weight reduction in women.

Before weight loss, no significant correlation was observed between plasma OC levels and skeletal muscle enzyme activities in either sex \((-0.41 < r < 0.35; \text{NS})\) (not shown). As shown in Table 3, no significant relationship was observed between the change in the marker enzyme of glycolysis (PFK) and changes in plasma OC levels \((-0.31 < r < 0.10; \text{NS})\). We found that the greater the increase in plasma OC levels, the greater the decrease in marker enzymes of skeletal muscle oxidative capacity (HADH, CS, COX) was in men \((-0.75 < r < -0.50, P < 0.05) \) but not in women \([-0.33 < r < 0.33, \text{not significant (NS)}]\) (Table 3). To verify whether the previous significant relationships in men were independent of changes in body weight, partial correlations were performed. Significant negative partial relationships were observed between variations in HCB, Aroclor 1260, and PCB 153 levels and changes in HADH, CS, and COX activities \((-0.68 < \text{partial } r < -0.48, P < 0.05) \), as shown in Table 4. Changes in HCB, Aroclor 1260, and PCB 153 levels and changes in HADH, CS, and COX activities also remained significantly correlated after correction for fat mass \((-0.70 < \text{partial } r < -0.50, P < 0.05) \) (not shown).

**DISCUSSION**

This study was undertaken to examine whether weight loss-induced increases in plasma pollutant levels were correlated to changes in determinants of the capacity for fatty acid utilization in human skeletal muscle. To our knowledge, this is the first study to report clear associations between variations in environmental pollutants and those in skeletal muscle markers of oxidative capacity in humans. Indeed, we showed that increased plasma OC levels were associated with decreased skeletal muscle oxidative enzyme activities (HADH, CS, and COX) in men even after control for body weight loss. No significant relationship was observed between changes in plasma OC and muscle oxidative capacity in response to weight loss in women.

A decreased reliance on lipid oxidation has previously been highlighted as a metabolic predictor of weight gain \( (29) \), and this has also been related to a decrease in skeletal muscle lipoprotein lipase activity \( (6) \), an enzyme promoting the uptake of free fatty acid in muscle after its catabolic action on circulating triglyceride-rich lipoproteins. Recent studies have supported the proposal that decreased oxidative enzyme capacity of skeletal muscle might be responsible for reduced fasting rates of fatty acid oxidation in obesity \( (11, 25) \). In these previous studies, it was also reported that a dietary restriction aiming at weight loss without change in baseline levels of physical fitness did not correct the predisposition toward fat esterification of skeletal muscle in obesity. These authors suggested that this could be due to the parallel reduction observed in CS and COX activities, two markers of the mitochondrial oxidative capacity. Although only associative in nature rather than truly mechanistic, the findings of the current study suggest that a rise in OC levels could be a plausible mechanism whereby the

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**Table 3. Relationships between changes in plasma OC levels and those in skeletal muscle enzyme activities in obese men and women in response to weight loss**

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \Delta \text{PFK} )</td>
<td>( \Delta \text{HADH} )</td>
<td>( \Delta \text{CS} )</td>
<td>( \Delta \text{COX} )</td>
<td>( \Delta \text{PFK} )</td>
<td>( \Delta \text{HADH} )</td>
<td>( \Delta \text{CS} )</td>
<td>( \Delta \text{COX} )</td>
</tr>
<tr>
<td>( p,p' )-DDE</td>
<td>-0.31</td>
<td>-0.54*</td>
<td>-0.50*</td>
<td>-0.51*</td>
<td>0.07</td>
<td>-0.25</td>
<td>-0.21</td>
<td>-0.14</td>
</tr>
<tr>
<td>( \beta )-HCH</td>
<td>-0.27</td>
<td>-0.58†</td>
<td>-0.39</td>
<td>-0.54*</td>
<td>-0.12</td>
<td>-0.11</td>
<td>-0.12</td>
<td>0.33</td>
</tr>
<tr>
<td>HCB</td>
<td>-0.13</td>
<td>-0.69‡</td>
<td>-0.54*</td>
<td>-0.58*</td>
<td>0.04</td>
<td>-0.33</td>
<td>-0.12</td>
<td>-0.24</td>
</tr>
<tr>
<td>Aroclor 1260</td>
<td>-0.25</td>
<td>-0.74‡</td>
<td>-0.64†</td>
<td>-0.71‡</td>
<td>0.10</td>
<td>-0.27</td>
<td>-0.17</td>
<td>-0.15</td>
</tr>
<tr>
<td>PCB 153</td>
<td>-0.28</td>
<td>-0.75‡</td>
<td>-0.65†</td>
<td>-0.69†</td>
<td>0.09</td>
<td>-0.24</td>
<td>-0.09</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

OC, organochlorine compounds; \( \Delta \), changes; DDE, \( 2,2' \)-bis(chlorophenyl)-1,1-dichloroethene; \( \beta \)-HCH, \( \beta \)-hexachlorocyclohexane; HCB, hexachlorobenzene; PCB, polychlorinated biphenyls. Statistical significance at *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \).

**Table 4. Relationships between changes in plasma OC levels and those in skeletal muscle oxidative enzyme activities corrected for changes in body weight in men in response to weight loss**

<table>
<thead>
<tr>
<th></th>
<th>( \Delta \text{HADH} )</th>
<th>( \Delta \text{CS} )</th>
<th>( \Delta \text{COX} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p,p' )-DDE</td>
<td>-0.37</td>
<td>-0.33</td>
<td>-0.33</td>
</tr>
<tr>
<td>( \beta )-HCH</td>
<td>-0.46</td>
<td>-0.29</td>
<td>-0.41</td>
</tr>
<tr>
<td>HCB</td>
<td>-0.65†</td>
<td>-0.48*</td>
<td>-0.51*</td>
</tr>
<tr>
<td>Aroclor 1260</td>
<td>-0.67†</td>
<td>-0.56*</td>
<td>-0.63*</td>
</tr>
<tr>
<td>PCB 153</td>
<td>-0.68‡</td>
<td>-0.56*</td>
<td>-0.60*</td>
</tr>
</tbody>
</table>

Statistical significance at *\( P < 0.05 \), †\( P < 0.01 \), and ‡\( P < 0.001 \).
capacity for fatty oxidation in muscle remained unchanged after weight loss, at least in men. This observation is concordant with previous data that have shown an impaired mitochondrial bioenergetic capacity in animals exposed to PCB (17, 19). In the current study, the specific impact on the oxidative capacity of skeletal muscle also appeared to be reinforced by the absence of a significant relationship observed between changes in OC levels and those in PFK activity, an enzyme of the glycolytic system.

It is not immediately clear why changes in pollutants and those in oxidative enzyme activities in response to weight loss were not significantly correlated in women. This absence of relationship may be due to the smaller increase in plasma OC levels in response to weight loss observed in women as opposed to men, as previously suggested (10). This could imply that a certain weight loss-induced increase in threshold of plasma OC levels is requisite for altering skeletal muscle oxidative capacity. Because of their lipophilicity, one could also hypothesize that a larger remaining fat mass in women as opposed to men after the weight loss program may render OC sequestration more favorable, thus dampening their mobilization. Further studies are required to elucidate this issue.

We have recently observed that weight loss-induced rise in OC levels was associated with decreased serum triiodothyronine (T₃) and resting metabolic rate (21). Thyroid hormones are well recognized to act as major regulators of oxidative energy metabolism at the level of the mitochondria. Short et al. (23) recently showed that T₃ increased oxidative skeletal muscle enzyme activities (CS and COX) in rodents. On our retrospective analyses, we did not observe any relationship between changes in serum thyroid hormone levels and changes in markers of muscle oxidative capacity. Bearing in mind that the effect of thyroid hormone on mitochondrial oxidative capacity is regulated through the thyroid hormone receptor, it is also biologically plausible that OC may alter oxidative muscle enzyme activities because of their known interaction with the human thyroid receptor (2). Although speculative, it is likely that these mechanisms are triggered in response to weight loss to promote weight regain, which in turn would prevent further release of OC into the circulation as a result of the redistribution of OC to adipose tissue. The absence of change in fasting pattern of fat oxidation by muscle in response to weight loss recently reported (11) could also be a mechanistic strategy taken by the myocyte to preserve its TG content for local buffering of OC. This assumption would be concordant with a recent report on a subsample of the present study, from which we observed that intramyocellular lipid concentration was not significantly reduced after weight loss (14).

In summary, the current study indicates that an increase in plasma OC levels derived from a weight loss is likely to be associated with a reduction in determinants of the capacity for fatty acid utilization in skeletal muscle. This finding is supported by the fact that an increase in OC levels is correlated with a decrease in HADH, CS, and COX activities in response to weight loss, at least in men. Further studies are, however, needed to verify whether these observations are causally related. Together, these intervention data suggest that environmental pollutants may be involved in the fatty acid metabolism perturbations commonly reported in human skeletal muscle in response to weight loss.

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REFERENCES


