Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene

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Submitted 2 July 2004; accepted in final form 7 October 2004

Jandacek, Ronald J., Nicole Anderson, Min Liu, Shuqin Zheng, Qing Yang, and Patrick Tso. Effects of yo-yo diet, caloric restriction, and olestra on tissue distribution of hexachlorobenzene. Am J Physiol Gastrointest Liver Physiol 288: G292–G299, 2005. First published October 28, 2004; doi:10.1152/ajpgi.00285.2004.—Chlorinated hydrocarbons are lipophilic, toxic, and persistent in the environment and animal tissues. They enter the body in food and are stored in adipose tissue. Loss of body fat through caloric restriction mobilizes stored lipophilic xenobiotics and results in distribution to other tissues. We have studied the reversibility of this process in mice that followed a regimen of body weight cycling. Weight gain was followed by weight loss, a second gain, and a second loss (“yo-yo diet regimen”). We measured the distribution of orally gavaged [14C]hexachlorobenzene, which is sparingly metabolized. We found that weight cycling has different effects in different organs. Continued weight loss resulted in a threefold increase of 14C amount and concentration in the brain. After weight regain, 14C in the brain decreased but then increased again after a second weight loss. Weight loss resulted in an increase in the concentration of 14C in adipose tissue without changing the total amount in that tissue. Weight loss and regain resulted in an increase in the concentration of 14C in the liver, which reflected an increase of fat in the liver. The regimen of weight gain and loss was repeated in mice gavaged with [14C]hexachlorobenzene, with one group receiving the nonabsorbable fat olestra in the diet. Combined dietary olestra and caloric restriction caused a 30-fold increase in the rate of excretion of 14C relative to an ad libitum diet or a reduced caloric diet alone. Distribution of 14C into the brain resulting from the restricted diet was reduced by 50% by dietary olestra.

body weight; xenobiotics; lipophilic; cycling; high fat

TOXIC LIPOPHILIC SUBSTANCES are widespread throughout the environment and the biosphere. The toxicity and/or carcinogenicity of many of these compounds has been extensively studied. Many are resistant to degradation and persist in the environment and in living organisms for long periods of time. They are known to ascend the food chain, and measurable levels have been found in people throughout all countries (2, 25). One group of these substances, comprising halogenated hydrocarbon pesticides and industrial byproducts, has been the focus of a 2001 United Nations-sponsored treaty that bans or restricts their use in the preparation of snack foods in the United States. The triacylglycerol components of adipocytes act as a sink into which the lipophiles partition. Other organs also retain some of the materials, but the principal storage site for the most lipophilic substances is adipose tissue. Other organs also retain some of the materials, but the principal storage site for the most lipophilic substances is adipose tissue.

Many lipophilic compounds are excreted into the intestine via biliary and nonbiliary paths, and a portion is reabsorbed from the intestine as part of an enterohepatic circulation (1, 11, 24). Studies of ways to intervene in the elimination of lipophiles have focused on interference with enterohepatic circulation with nonabsorbable dietary substances that adsorb or dissolve intestinal lipophiles and decrease their reabsorption.

Materials

Materials

[14C]hexachlorobenzene (0.45 μCi/μg) was purchased from Sigma-Aldrich (St. Louis, MO). Olestra was supplied by Procter & Gamble (Cincinnati, OH). Pelleted high-fat diet was formulated (Dyets, Bethlehem, PA) with 40% of energy fat (95–5 butterfat, soybean oil), 15% protein, and 46% carbohydrate, and required micronutrients. The pelleted diet containing olestra was formulated (Research Diets, New Jersey.

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Brunswick, NJ) with the same macronutrient composition as the high-fat diet, required micronutrients, and 10% by weight olestra (Procter & Gamble). This diet also included additional vitamins A, E, D, and K (as menadione) to compensate for reduced absorption of these vitamins by the olestra.

**Animals and Diets**

The protocols for all studies were approved by the Institutional Animal Care and Use Committee.

**Study 1, weight cycling.** The study was carried out with 6-wk-old male C57BL/6 mice (Taconic, Germantown, NY). The animals were allowed to acclimate to the environment of the room for 1 wk. The room temperature was 21°C with a 12:12-h light-dark cycle. Initially, each mouse was caged individually and provided tap water and chow ad libitum. The animals were assigned into nine groups of eight according to body weights and acclimated to the high-fat diet for 5–6 days. All animals were gavaged with 0.7 μCu of [14C]hexachlorobenzene dissolved in soybean oil using a feeding needle. To facilitate the necropsy and tissue collection scheduled in the study, four groups (groups 1, 3, 6, 8) received the gavage on day 5 and the remaining groups on day 6. Body weights were measured at the times of gavage, death, and changes of diet regimens. The treatment of the groups is presented graphically in Fig. 1A. Group 1 remained on the ad libitum high-fat diet for 15 days; the animals were then killed, and tissues were harvested. After 15 days of the ad libitum regimen, groups 5-9 began a 55% reduction (calculated from 3 days of food consumption during ad libitum feeding) in food intake. Groups 2-4 continued the ad libitum diet. After 5 days with these regimens, groups 2 (fed ad libitum) and 5 (diet restricted) were killed and tissues collected. Groups 6 and 7 returned to the ad libitum high-fat diet. After 5 days, groups 3, 6, and 8 were killed for tissue collection. Group 4 continued the ad libitum diet for 5 days, and groups 7 and 9 received 55%-restricted diet until death and tissue collection after 5 days.

**Study 2, reduction in enterohepatic cycling.** The animals were of the same source and strain as described above and were acclimated and housed as in study 1. Animals were assigned by body weights into four groups of eight each. Each mouse was caged individually and provided tap water and chow ad libitum. Animals were fed the high-fat diet ad libitum and initial body weights were measured. On day 4, animals were gavaged with 1.0 μCu of [14C]hexachlorobenzene dissolved in soybean oil using a feeding needle. Body weights were measured on the day of gavage. The treatment of the groups is described in Fig. 1B. Group 1 remained on the ad libitum high-fat diet for 20 days, and the animals were then killed and tissues were harvested. On day 20, groups 3 and 4 began a 50% caloric reduction (based on 3 days of measured ad libitum food consumption; olestra was assumed to provide no utilizable energy). Group 4 switched from eating the high-fat diet to a 50% caloric reduction diet made with 10% olestra. On day 35, tissues were collected from groups 2–4. Body weights were measured for all animals on the day of necropsy.

**Fecal Collections**

A total fecal collection was made on the 2 days after the day of gavage of [14C]hexachlorobenzene in study 1. The fecal matter was weighed and homogenized with 20 μl of Millipore water, and aliquots were assayed by combustion and scintillation counting as described in Tissue Analysis. In study 2, a total fecal collection was performed on days 24 and 25 and then on days 30 and 31. The fecal matter was analyzed as in study 1.

**Necropsy**

The animals were anesthetized with ketamine and xylazine. Blood was taken from the descending aorta. Livers, brains, and epididymal fat pads were removed from all mice in both studies. Kidneys, hearts, and renal fat were also removed in study 1. The contribution of radioactivity from the blood in the tissues was found to be negligible relative to the concentration in the tissues, and measurements were made without exsanguination. Weights of total tissues were measured for all tissues and then two portions from each tissue were taken to assay radioactivity. Blood was spun at 10,000 g, and total plasma was extracted. Tissue and plasma samples were stored frozen at −20°C until analyzed.

**Tissue Analysis**

Tissues and feces were analyzed by oxidation and scintillation counting. Oxidation and conversion to carbon dioxide were carried out with the Harvey Biological Oxidizer OX700 (R. J. Harvey, Hillsdale, NJ). Tissues were burned at 900°C, and the radioisotope was captured as 14CO2 in 14C scintillation cocktail (R. J. Harvey). Two weighed portions were oxidized for each tissue collected from each animal. Plasma, which showed no signs of hemolysis, was inserted directly into the scintillation fluid (without oxidation). 14C activity in each vial was counted with a scintillation counter (Tri-Carb 1900 CA) and recorded as dpm. For all samples, quenching and efficiency were compensated using the counter’s quench indicating parameter from the spectra of a standard source.

**Liver Lipids**

Total fatty acid-based fat in livers was quantified by saponification and methylation of tissue samples to which a known mass of pentadecanoic acid had been added as an internal standard. The methyl esters were analyzed by gas chromatography as previously described (12).
Statistical Methods

ANOVA with Tukey’s test for significance was used to compare results with significance attained at a P value of < 0.05 (SigmaStat, Chicago, IL). Data are presented as means ± 1 SE. Correlations were determined from linear regression analyses (SigmaPlot, Chicago, IL).

RESULTS

Study 1, Weight Cycling

Design of the study is described by the diagram in Fig. 1A. Nine groups of 8 mice each were studied with varying periods of ad libitum and restricted dietary intake. The study was designed to determine the effects of weight cycling (groups 1, 5–7) and weight reduction (groups 5, 8, 9) compared with animals receiving diet ad libitum with concomitant weight increase.

Body Weights and Tissue Weights

Body weights for each group are presented in Fig. 2. Groups 1–4 continued to gain weight during the study. As expected, groups 5–9 gained weight during ad libitum feeding and lost weight during diet restriction.

The weights of tissues that were completely excised are presented in Table 1. Weights of the brains did not vary with changes in caloric intake. Weight of the epididymal fat pad increased with ad libitum feeding of the high-fat diet (group 4) and decreased with caloric restriction (groups 8, 9). Weights of the livers decreased after caloric restriction (groups 5, 8, 9), markedly increased after the return to ad libitum diet after a period of caloric restriction (group 6), and decreased again after the second period of caloric restriction (group 7). The weights of the kidney decreased with caloric restriction (groups 5, 8, 9).

Tissue Radioactivity

The fecal collection from nine animals from the 2 days after gavage was assayed for radioactivity. Less than 1% of the dose was recovered in this collection, consistent with essentially complete absorption of the gavaged [14C]hexachlorobenzene.

The recovery of 14C in the liver is presented in Fig. 3 in terms of percent of dose per liver. The most striking increase was seen following the return of the ad libitum diet after the period of diet restriction (group 6) corresponding to the increase in liver weight also seen in this group (Table 1).

Refeeding resulted in an increase in liver lipids. The total fat (based on saponifiable fatty acids) in the liver after a restricted diet (group 5) was 26.4 ± 1.6 mg, which did not differ from 21.6 ± 1.4 mg in the livers after the restricted diet (group 5) and after second weight loss (26.4 ± 1.6, group 7). Liver fat in the group that maintained the ad libitum diet for the comparable period (group 3) was 50.7 ± 7.3 mg, which did not differ from group 6. Fat in the group that was killed at the time of study (groups 3 and 6, but which received the restricted diet (group 8), was signifi-
Correlation of hexachlorobenzene in total liver with the amount of fat per liver is shown in Fig. 4A. There was a positive correlation of hexachlorobenzene in the livers with the fat in the liver. The open squares in Fig. 4A represent the values for group 6, in which liver lipid and hexachlorobenzene were greater than the other groups.

Concentration in the fat pad reflected the size of the fat pad, decreasing with the fat pad mass (Fig. 4B). The concentration in the renal fat samples showed similar responses to diet seen in the epididymal fat pads (Table 2). The correlation of the concentration of hexachlorobenzene in the epididymal and renal fat is presented in Fig. 4C.

The amount of [14C]hexachlorobenzene in the epididymal fat pad did not change with diet regimen (range of 3.15–3.97% of dose/fat pad), with the exception of that seen after the continued diet restriction in group 9. The [14C]hexachlorobenzene in the fat pad in group 9 was significantly less than that of the other groups (1.95/60.26% of dose). However, the concentration of [14C]hexachlorobenzene in the epididymal fat pad increased with caloric restriction (groups 5, 8, 9) (Fig. 5). The concentration decreased following the return to the ad

Table 2. Concentration of hexachlorobenzene (HCB) in samples of muscle and renal fat (% dose/g tissue) and plasma (dpm/100 μl) in weight cycling study (study 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Muscle</th>
<th>Renal Fat</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.36±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.73±1.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54±4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.48±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.10±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43±3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.85±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37±2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1.01±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.96±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36±3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>1.74±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.91±1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50±4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>1.07±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.29±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52±2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>0.90±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.75±0.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38±2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>1.52±0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.20±0.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>56±8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>1.50±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.45±1.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>82±8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE. Common superscripts denote groups that are not different.

Fig. 4. A: percent of administered HCB dose/liver plotted with the total liver lipids from the weight-cycling study (study 1). B: Results from group 6 (weight regain). C: Results from remaining groups (r² = 0.50; P < 0.0001). B: concentration of HCB in the epididymal fat pad plotted against the total mass of the fat pad (r² = 0.49; P < 0.0001) (weight-cycling study, study 1). C: concentration of HCB in epididymal and renal fat pads (r² = 0.81; P < 0.0001) in the weight-cycling study (study 1).

Fig. 5. Concentration of HCB in the epididymal fat pads of animals in the weight-cycling study (study 1). Concentration (% dose/g tissue) is presented numerically for each group on the graph of the body weights and time of study. Common subscript letters denote groups that were not significantly different.

Diagram 1: Diagram showing the concentration of HCB in the epididymal fat pad of animals in the weight-cycling study (study 1). Concentration (% dose/g tissue) is presented numerically for each group on the graph of the body weights and time of study.

Diagram 2: Diagram showing the concentration of HCB in the renal fat pad of animals in the weight-cycling study (study 1). Concentration (% dose/g tissue) is presented numerically for each group on the graph of the body weights and time of study.

Diagram 3: Diagram showing the concentration of HCB in the muscle tissue of animals in the weight-cycling study (study 1). Concentration (% dose/g tissue) is presented numerically for each group on the graph of the body weights and time of study.

Diagram 4: Diagram showing the concentration of HCB in the plasma samples of animals in the weight-cycling study (study 1). Concentration (dpm/100 μl) is presented numerically for each group on the graph of the body weights and time of study.

Diagram 5: Diagram showing the concentration of HCB in the liver tissue of animals in the weight-cycling study (study 1). Concentration (% dose/g tissue) is presented numerically for each group on the graph of the body weights and time of study.
libitum diet after caloric restriction (group 6), and there was a trend toward an increase during the second period of caloric restriction (group 7).

The total amount of $^{14}$C in the brain increased with continuing caloric restriction (groups 5, 8, 9) as shown in Fig. 6. A decrease was observed following the resumption of the ad libitum diet after the period of weight restriction (group 6). A trend toward an increase in the level in the brain was seen after the second period of caloric restriction (group 7). Since there were no changes in brain weights with regimen (Table 1), the concentration of $^{14}$C in the brain changed in the same manner as the total recovery of $[^{14}$C]hexachlorobenzene in the brain.

$^{14}$C in the kidney increased with continued caloric restriction (groups 8 and 9) (Fig. 7). There was no significant change after regain of weight (group 6) or after the second period of caloric restriction (group 7).

The recovery of $^{14}$C in muscle is presented in terms of percent of dose per gram of tissue in Table 2. Although concentration apparently increased with time regardless of the dietary regimen, it was evident at necropsy that many of the muscle samples were contaminated with amounts of adipose tissue, which presumably prevented accurate measurements of concentration in adulterated muscle tissue.

The $^{14}$C in plasma was significantly higher in the animals that underwent caloric restriction for the entire study (group 9) (Table 2). There was an apparent trend toward higher counts among the groups that lost weight (groups 5, 8, 9) compared with those that gained weight (groups 2–4).

Study 2. Weight Loss and Reduction in Enterohepatic Circulation

The design of the study is presented in Fig. 1B. Animals were fed ad libitum a high-fat diet and then followed one of three dietary regimens: continued ad libitum ingestion, caloric restriction of the high-fat diet, or caloric restriction of the high-fat diet supplemented with olestra. The animals that received olestra exhibited no untoward effects, and fecal pellets were well formed with no sign of separation of the unabsorbed oil from the other fecal matter.

Body Weights and Tissue Weights

As expected, the body weights of the animals increased with ad libitum feeding and decreased during diet restriction. At time of death, the mean weight of group 2 (ad libitum feeding) animals was 33.0 ± 1.26 g, which was significantly greater than that of group 3 (diet restriction, 24.4 ± 0.75 g) and group 4 (diet restriction plus olestra; 23.4 ± 0.69 g). The weights of groups 3 and 4 were not different.

Weights of the epididymal fat pads are presented in Table 3. The fat pads increased in weight with ad libitum feeding and decreased after caloric restriction. There was no difference between the fat pad weights of groups 3 and 4, which received a calorically restricted diet with or without olestra, respectively. Liver weights in the calorically restricted groups were

Table 3. Tissue weights from study of interruption of enterohepatic cycling and weight loss on HCB tissue distribution (study 2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Epididymal Fat Pad</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.31 ± 0.08a</td>
<td>0.52 ± 0.05a</td>
<td>0.42 ± 0.02a</td>
</tr>
<tr>
<td>2</td>
<td>1.45 ± 0.07a</td>
<td>0.73 ± 0.04b</td>
<td>0.41 ± 0.004a</td>
</tr>
<tr>
<td>3</td>
<td>0.74 ± 0.04b</td>
<td>0.30 ± 0.04b</td>
<td>0.39 ± 0.01a</td>
</tr>
<tr>
<td>4</td>
<td>0.66 ± 0.04b</td>
<td>0.25 ± 0.04c</td>
<td>0.39 ± 0.01a</td>
</tr>
</tbody>
</table>

Values are means ± SE. Common superscripts denote groups that are not different.
significantly less than those of animals fed ad libitum. The weights of the brains (Table 3) did not differ among groups.

**Tissue Radioactivity**

Total recovery of $^{14}$C in the liver is presented in Fig. 8. $^{14}$C in the livers from the animals that received olestra with caloric restriction was significantly less than that of the animals fed ad libitum or with caloric restriction. The concentration of $^{14}$C in the liver was less in the olestra-fed animals (0.44 ± 0.03% dose/g) than in the diet-restricted group (0.78 ± 0.03% dose/g). The concentration in liver in the olestra group did not differ from that in the ad libitum group (0.43 ± 0.02% dose/g, group 2).

The amount of $^{14}$C in the epididymal fat pad was reduced relative to the other three groups after the period of the caloric restriction and olestra feeding (Fig. 9). The concentration in the fat pad decreased with continued ad libitum feeding and increased with caloric restriction. The concentration of $^{14}$C in the fat pad was negatively correlated with the mass of the fat pad for groups 1–3 ($r^2 = 0.67; P < 0.0001$). The concentration in group 4 (caloric restriction plus olestra; 7.89 ± 0.58% dose/g) was significantly less than that of group 3 (caloric restriction; 10.86 ± 1.12%) and significantly greater than that of the group 2 (ad libitum feeding; 4.70 ± 0.35%).

$^{14}$C in the brain increased with caloric restriction (Fig. 10). The amount in the olestra-fed animals was less than that of the group with caloric restriction alone and greater than that of the animals fed ad libitum.

$^{14}$C in plasma was 44 ± 4 dpm/100 µl in the group that received the diet ad libitum. As we observed in the weight-cycling study, this concentration was significantly less than that of the calorically restricted group (87 ± 10 dpm/100 µl), which did not differ from the group that received olestra alone with caloric restriction (69 ± 6 dpm/100 µl).

**Fecal Excretion**

Two 48-h fecal collections were taken from all animals on days 5–6 and 11–12 after the beginning of the restricted diets. Results are shown in Fig. 11. There was no difference in the

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**Fig. 8.** Recovery of HCB in the livers of animals in the enterohepatic-cycling study (study 2). The recovery/liver (%dose/total liver) is presented numerically for each group on the graph of the body weights and time of study. Common subscript letters denote the groups that were not significantly different.

**Fig. 9.** Recovery of HCB in the epididymal fat pads of animals in the enterohepatic-cycling study (study 2). The recovery/fat pad (%dose/total fat pad) is presented numerically for each group on the graph of the body weights and time of study. Common subscript letters denote the groups that were not significantly different.

**Fig. 10.** Recovery of HCB in the brains of animals in the enterohepatic-cycling study (study 2). Recovery/liver (%dose/total brain) is presented numerically for each group on the graph of the body weights and time of study. Common subscripts denote the groups that were not significantly different.
rate of excretion between the animals fed ad libitum and those that were energy-restricted. Animals fed olestra, however, excreted 30-fold higher levels than other groups.

**DISCUSSION**

Results from these studies provide insight into the interaction of changes in the size of the body adipose depots and the distribution of lipophilic toxins to tissues. This distribution is affected by changes in body weight and is influenced by interference with enterohepatic circulation.

A dieting regimen was used to mimic a low level of exposure to organochlorine in foods. Fat pads at the completion of the caloric reduction regimen were not entirely depleted. Concentration of hexachlorobenzene in adipose tissue increased, but the total amount of hexachlorobenzene in the epididymal fat pad remained essentially constant during caloric restriction. Similar concentrations of hexachlorobenzene in renal fat and epididymal fat suggest that hexachlorobenzene deposition in fat is independent of the site, although epididymal and perirenal preadipocytes from the rat have been reported to differ in their uptake of lipid (3).

In terms of mass and percentage of total body burden, the amounts of hexachlorobenzene in the brain and kidney were small and presumably predict minimal health risk if the body burden of hexachlorobenzene is small. Distribution of hexachlorobenzene into these organs is consistent with the caloric deficit placing the adipose stores in a negative balance with increased lipolysis. During caloric deprivation, hexachlorobenzene would then be released along with the fatty acids and decreased circulating lipoproteins, thus suggesting the importance of free fatty acids in carrying hexachlorobenzene. The distribution of hexachlorobenzene among plasma fractions after fasting will be addressed in future studies.

Although organochlorine compounds are very lipophilic, there is evidence that they are transported rapidly out of triacylglycerol during lipolysis. Vost and Maclean (27) reported 1,1,1-trichloro-2,2-bis(p-chlorophenol)ethane to be cleared more rapidly from chylomicrons than triacylglycerol. We have observed similar behavior for hexachlorobenzene (data not shown here). These observations suggest an affinity of organochlorines for the fatty acids generated at the water-triacylglycerol interface during lipolysis. How the plasma carriers facilitate hexachlorobenzene entry into the brain is an interesting question with potential physiological implications.

The high level of hexachlorobenzene seen in the livers of animals that underwent weight regain is consistent with deposition of fat in the liver after resumption of ad libitum feeding. The liver lipids and hexachlorobenzene in the refed animals were significantly higher than those from the animals that were on the ad libitum diet throughout the study. An interpretation of the data is that fasting and refeeding resulted in the liver: 1) taking up the circulating lipids in the blood; 2) accelerating synthesis of triacylglycerol despite the fact that there are more circulating triacylglycerols during ad libitum feeding; and 3) reducing oxidation of the liver lipids or secreting less very low-density lipoproteins. Resumption of an ad libitum diet after a period of starvation was previously reported to result in an increase in hepatic lipid synthesis and deposition in rats (5). The relationship of fasting and refeeding to liver lipid deposition is currently being studied in our laboratory.

Introduction of olestra into the diets of the energy-restricted animals resulted in a 30-fold increase in the excretion of hexachlorobenzene to more than 1% of the gavaged dose per day. One interpretation of this result is that more than 1% of the stored hexachlorobenzene appeared in the small intestine each day, of which essentially all was reabsorbed regardless of the caloric balance of the animal. Dietary olestra provided a lipophilic sink that interfered with the enterohepatic circulation of hexachlorobenzene by dissolution and transport into the feces. Another explanation for the increased excretion with olestra is that olestra in the intestinal lumen increased transport of hexachlorobenzene into the lumen. Because olestra probably does not stimulate bile flow (13, 16), this process would require direct secretion from the enterocyte in a nonbiliary excretion (11, 24). It is indeed possible that the sequestration of hexachlorobenzene in the intestinal lumen increased the concentration gradient from the enterocyte to the lumen and thereby enhanced transport. These possibilities suggest further study to elucidate the process. Regardless of the mechanism, the combination of dietary olestra with caloric restriction appears to be an effective way to significantly increase the excretion rate and thereby reduce the body burden and tissue concentration of organochlorine compounds.

Because the primary focus of study 2 was the effect of olestra on distribution into the brain during caloric restriction, we did not include a group that received olestra during ad libitum feeding. A previous study (20) found that caloric

**Fig. 11. Recovery of 14C in a 48-h collection of feces from mice gavaged with HCB and fed either ad libitum, restricted diet intake, or restricted diet intake with olestra. Common letters denote values that were not different.**
restriction increased the effect of olestra on organochlorine excretion. The rate of DDE excretion in gerbils fed olestra in combination with caloric deprivation was more than that from animals fed olestra and ad libitum diet. Also, an earlier study of 8% dietary olestra in ad libitum feeding of rats with hexachlorobenzene resulted in a 2.5-fold increase in fecal excretion relative to the control group during 3 wk of ad libitum feeding (23), markedly less than the 30-fold increase we observed with olestra and caloric restriction.

Two studies (9, 19) of the effect of olestra on organochlorine compounds (dioxins) in humans followed protocols that did not alter caloric intake. However, a case report of a patient with a very high body burden of PCBs who was treated with olestra underwent a weight reduction from 100 to 80 kg during the treatment period (T. Redgrave, personal communication). This regimen was accompanied by a marked reduction in the concentration of PCBs in adipose biopsies.

Study of hexachlorobenzene both answers and raises questions about similar studies with other organochlorine compounds. Hexachlorobenzene is sparingly metabolized (primarily to pentachlorophenol) (14) and therefore remains in a highly lipophilic form. One might predict that the lipophilic parent compounds of some congeners of PCBs, dioxins, and other lipophilic materials would behave in a manner like PCB during “yo-yo” diet cycling. The more polar metabolites of organochlorine compounds may undergo a different pattern of distribution during weight loss and regain, which is an important area to address in future studies.

We found that the only significant changes in plasma levels of hexachlorobenzene were seen after the maximum weight loss and fat loss attained in both studies. It is possible, however, that the low amount of radioactivity in the plasma samples may have prevented us from detecting small changes in concentration that accompanied lesser weight loss.

Inclusion of olestra in the diet resulted in interruption of enterohepatic circulation of hexachlorobenzene and reduction in all tissues even during the periods of caloric restriction. These observations suggest that an appropriate regimen for the removal of lipophilic toxins is the combination of caloric restriction and the interruption of enterohepatic circulation with a nonabsorbable fat. Presumably, the use of a lipase inhibitor, such as orlistat, will also result in partial blockage of the enterohepatic circulation of lipophiles by providing an undigested intestinal triacylglycerol phase that will solubilize these compounds.

GRANTS

This work was supported by U. S. Department of Agriculture Grant 02–00824 and National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-56910 and DK-59630.

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