One-Day Dietary Restriction Changes Hepatic Metabolism and Potentiates the Hepatotoxicity of Carbon Tetrachloride and Chloroform in Rats

LI-QIANG QIN,1 YUAN WANG,2 JIA-YING XI,1 TAKASHI KANEKO,3 AKIO SATO3 and PEI-YU WANG4

1Department of Nutrition and Food Hygiene, School of Radiation Medicine and Public Health, Soochow University, Suzhou, China
2Internal Medicine, Fourth Affiliated Hospital, Hebei Medical University, Shijiazhuang, China
3Department of Environmental Health, School of Medicine, Yamanashi University, Chuo, Japan
4Department of Social Medicine and Health Education, School of Public Health, Peking University, Beijing, China

QIN, L.-Q., WANG, Y., XI, J.-Y., KANEKO, T., SATO, A. and WANG, P.-Y. One-Day Dietary Restriction Changes Hepatic Metabolism and Potentiates the Hepatotoxicity of Carbon Tetrachloride and Chloroform in Rats. Tohoku J. Exp. Med., 2007, 212 (4), 379-387 —— Although dietary restriction (DR) is common in modern society, research about hepatic metabolism and the hepatotoxicity induced by DR has been conducted less intensively than that induced by fasting. In the present study, we fed male Wistar rats at five levels of food intake for one day, including conventional feeding (60 kcal), three of DR (45, 30, and 15 kcal), and fasting (0 kcal), and observed the metabolic changes of hepatic cytochrome P450 2E1 (CYP2E1) and the hepatotoxicity of chloroform (CHCl3) and carbon tetrachloride (CCl4). The CYP2E1 content was significantly increased in 15 kcal-food and fasting groups. The hepatic glutathione (GSH) content, which protects the liver from hepatotoxic agents, was depleted in 15 kcal-food and fasting groups. After the challenge by CHCl3 and CCl4, the activities of aspartate aminotransferase and alanine aminotransferase, marker enzymes for liver damage, were elevated remarkably at all food groups. Moreover, their activities increased significantly in DR groups, in comparison to the corresponding 60 kcal-food group. After the challenge, the hepatic GSH content was also depleted significantly in 15 kcal-food and fasting groups. CHCl3 was cleared by hepatic metabolism about 8-10 times faster than that of CCl4. Similarly, the areas under the blood concentration-time curve of CCl4 was as much as twice that of the corresponding CHCl3. In conclusion, when food was restricted to less than half of conventional amount, hepatic metabolism was affected and the hepatotoxicity induced by CCl4 or CHCl3 was augmented by, at least in part, CYP2E1 induction and GSH depletion. ——— dietary restriction; hepatotoxicity; hepatic cytochrome P450 2E1; chloroform; carbon tetrachloride © 2007 Tohoku University Medical Press
Fasting is a measure occasionally used in clinical medicine for diagnostic and therapeutic purposes. It is well-known that fasting potentiates the hepatotoxicity of chemicals, such as carbon tetrachloride (CCl₄), which is metabolically activated by cytochrome P450 (CYP) in hepatic microsomes (Hong et al. 1987; Imaoka et al. 1990; Ueng et al. 1993; Wang et al. 1995; Sheweita et al. 2001). CYP2E1 is the most important isoform enhanced by fasting (Hong et al. 1987; Imaoka et al. 1990). Although fasting is very rare, dietary restriction (DR) is more common due to health reasons in developed countries or for economic reasons in some developing countries, or simply as a method of dieting for esthetic reasons. It has been demonstrated that DR modulates many fundamental physiological processes, such as drug metabolism and hormonal regulation, and increases laboratory rat survival (Keenan et al. 1996; Duffy et al. 1997; Frame et al. 1998; Hubert et al. 2000). However, animals are at an increased risk of dying from malnutrition if their dietary intake is extremely low (Frame et al. 1998). Although DR is considered to influence the susceptibility to toxic effects of chemicals such as CCl₄ and chloroform (CHCl₃), the research about how much DR can affect the hepatic metabolism and hepatotoxicity has not been intensively conducted.

In previous studies, researchers have always observed the effects of the duration of fasting (from 12-hr to 72-hr) on the hepatic metabolism and hepatotoxicity (Nakajima et al. 1982; Hong et al. 1987; Imaoka et al. 1990; Ueng et al. 1993; Wang et al. 1995). To our knowledge, there have been no reports that have designated the amount of food intake at five levels including conventional feeding (60 kcal), three of DR (45, 30 and 15 kcal) and fasting (0 kcal). In the present study, we comprehensively observed the metabolic changes of the hepatic CYP content, especially the content and activity of CYP2E1, at five levels of food intake. Both CHCl₃ and CCl₄ were used as hepatotoxicants. Thus, we have the chance to observe their differences, if any, in the case of DR.

**MATERIALS AND METHODS**

*Animals and pretreatment*

One hundred and seventy-five male Wistar rats (8-week old) in SPF status were purchased from Beijing Vital River Laboratory Animal Company (Beijing, China) and kept individually in stainless steel wire-bottomed cages in an air-conditioned room (22 ± 2°C, 55 ± 10% relative humidity) with artificial lighting switched on from 06:00 to 18:00. They were maintained on commercial powder food (Beijing Laboratory Animal Research Center, Beijing, China) and water *ad libitum*. A few days’ observation revealed that the food intake of rats is about 18 ± 1.6 g/day/rat on average, corresponding to about 60 kcal. Thus, the food intake levels were assigned to be 0, 4.5, 9, 13.5 and 18 g/rat (corresponding to 0, 15, 30, 45, and 60 kcal), respectively. At 10 weeks of age, the animals were fed the designated powder food described above for one day. At 10:00 on the next day, the rats were either killed for the determination of CYP and metabolism study in vitro, or challenged orally with CHCl₃/CCl₄ for the toxicokinetics studies in vivo. The study design is shown in Fig. 1. The experiments were performed in accordance with the Guidelines for Animal Experiments, Peking University and Soochow University.

*Measurements of hepatic glutathione (GSH) content, microsomal protein, and total CYP contents*

Five rats from each group were killed by decapitation at 10:00 and their livers were removed. Liver homogenate (25% w/v in 1.15% KCl-0.01 M phosphate buffer, pH 7.4) was centrifuged at 10,000 g at 0°C for 20 min. A portion of the supernatant (0.5 ml) was used for the measurement of GSH content according to the method of Ellman (1959). The remaining supernatant was further centrifuged at 105,000 g at 4°C for 60 min to harvest the microsomal pellets. The pellets were suspended in the phosphate buffer and recentrifuged at 105,000 g at 4°C for 60 min. The washed microsomal pellets were resuspended with the buffer and the protein content was measured according to the method of Lowry (1951). The protein content was then adjusted to a concentration of 10 mg/ml with the buffer solution, and the suspension was stored at –80°C until use. Microsomal CYP content was determined spectrophotometrically according to the method of Omura and Sato (1964).
Measurements of Hepatic CYP2E1 protein and activity

Liver microsomes were separated electrophoretically on a sodium dodecyl sulfate-polyacrylamide gel (10%) and the separated CYP isozymes were transferred to a nitrocellulose membrane. After exposure to a polyclonal antibody (goat anti-rat CYP2E1 serum, Dai-ichi Pure Chemicals, Tokyo) for 2 hrs at room temperature, the isozymes were analyzed for CYP2E1 by immunostaining with a rabbit anti-goat IgG alkaline phosphatase conjugate (Sigma Chemicals, St. Louis, MO, USA) and a NBT/BCIP kit (Pierce, Rockford, IL, USA). The staining density of the CYP2E1 band was quantified with a densitometer (Arcus II, Agfa, Wilmington, MA, USA) using the built-in software program (Quantity One, Huntington Station, NY, USA). The result of the immunquantitation was expressed in arbitrary units relative to the amount of CYP2E1 protein (Qin et al. 2005).

The activity of hepatic CYP2E1 was assessed by measuring the demethylation rate of dimethylnitrosamine (DMN) as described by Wang (1999). The DMN demethylation reaction mixture (1.0 ml) contained a final concentration of 0.07 mM EDTA, 0.2 mM NADP, 14 mM MgCl₂, 215 mM KCl, 70 mM Tris-HCl buffer (pH 7.4), 10 mM isocitrate, 0.3 units of isocitrate dehydrogenase and 1.0 mM DMN in addition to the microsomes corresponding to 0.5 mg protein. The mixture was incubated at 37°C for 30 min.

CHCl₃ and CCl₄ metabolism in vitro

The metabolism of CHCl₃ and CCl₄ in vitro was assessed by measuring the rate of substrate disappearance according to a method described previously (Kaneko et al. 1993). The reaction mixture (0.5 ml) contained a final concentration of 1.0 mM NADP, 50 mM MgCl₂, 20 mM glucose 6-phosphate (G-6-P), 50 mM K/K-phosphate buffer, and 0.25 mM CHCl₃ or 0.25 mM CCl₄ in addition to 2 units of G-6-P dehydrogenase and microsomes corresponding to 1.0 mg protein.

Administration and toxicokinetic study of CHCl₃ and CCl₄

At 10:00 on the day of the experiment, 10 rats of each group were orally administered CHCl₃ or CCl₄ at a dose of 3.35 mmol/kg in corn oil (2 ml/kg). The other 10 rats received corn oil only as the control group. After administration, a small blood sample (20 μl) was taken with a heparinized micropipette from a cut in the tail at preselected intervals (0, 4, 8, 12, 16, 20, and 24 hrs) and the blood CHCl₃ or CCl₄ concentration was measured according to the method of Wang (Wang et al. 1997). The areas under the blood concentration-time curve (AUC) of CHCl₃ and CCl₄ were calculated using the trapezoidal rule from 0 to 24 hrs.

Biochemical examination

Twenty-four hrs after CHCl₃ or CCl₄ administration,
rats were killed by decapitation and the blood was collected and then centrifuged at 1,000 g for 5 min. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined with commercial test kits (Wako Pure Chemicals, Osaka) as indices of liver injury. The GSH content in the liver was measured according to the method of Ellman (1959).

**Statistics**

Data were subjected to ANOVA with the help of StatView 4.0 (Abacus Concepts, Berkeley). Fisher’s protected least significant difference (PLSD) test was used when there was a significant difference among the groups. A p value of 0.05 was used as the criterion of significance.

**RESULTS**

The body weight showed a slight fall in 45 kcal-food and 30 kcal-food groups, and further DR (15 kcal-food and fasting groups) resulted in the significant decrease of body weight. The liver weight was more sensitive to DR because the significant decrease started from 30 kcal-food group. The liver weight as a fraction of body weight (%)

| Table 1. Effect of food restriction on body weight and liver weight of rats. |
|----------------------------------|------------------|----------------|----------------|----------------|----------------|
| Food intake (kcal)               | 60 (Control)     | 45             | 30            | 15            | 0             |
| Body weight (g)                  | 255 ± 4          | 251 ± 7        | 246 ± 9       | 240 ± 10 a    | 227 ± 5 a     |
| Liver weight (g)                 | 10.8 ± 0.6       | 10.2 ± 0.7     | 9.3 ± 0.9 a   | 7.8 ± 0.8 a   | 6.4 ± 0.4 a   |
| LW/BW (%)                        | 4.2 ± 0.3        | 4.1 ± 0.2      | 3.6 ± 0.3 a   | 3.3 ± 0.3 a   | 3.0 ± 0.2 a   |

Values represent mean ± S.D. for 5 rats.
LW/BW, liver weight (g)/body weight (g).
*Significantly different from the 60 kcal-food group (controls) (p < 0.05).

| Table 2. Effect of food restriction on microsomal protein, CYP, CYP2E1, DMN and metabolic rate of CHCl₃ and CCl₄ in vitro. |
|---------------------------------------------------------------|------------------|----------------|----------------|----------------|----------------|
| Food intake (kcal)                                           | 60 (Control)     | 45             | 30            | 15            | 0             |
| Microsomal protein (mg/g liver)                              | 17.2 ± 2.5       | 17.5 ± 2.3     | 18.1 ± 2.1    | 18.8 ± 1.8    | 19.6 ± 2.3    |
| CYP (nmol/mg protein)                                        | 0.74 ± 0.06      | 0.79 ± 0.13    | 0.88 ± 0.20   | 0.90 ± 0.18   | 0.98 ± 0.12 a |
| CYP2E1 (arbitrary)                                           | 0.49 ± 0.05      | 0.50 ± 0.09    | 0.59 ± 0.08   | 0.82 ± 0.09 a | 0.99 ± 0.15 a |
| DMN (nmol/min/mg protein)                                   | 1.35 ± 0.23      | 1.42 ± 0.25    | 1.73 ± 0.24   | 2.20 ± 0.27 a | 2.85 ± 0.31 a |
| GSH (mg/g liver)                                             | 2.52 ± 0.33      | 2.47 ± 0.34    | 2.36 ± 0.24   | 2.02 ± 0.23 a | 1.95 ± 0.22 a |
| Metabolic rate of CHCl₃ (nmol/mg protein/min)                | 0.98 ± 0.22      | 1.13 ± 0.25    | 1.38 ± 0.28   | 2.39 ± 0.31 a | 2.97 ± 0.39 a |
| Metabolic rate of CCl₄ (nmol/mg protein/min)                 | 0.11 ± 0.02      | 0.10 ± 0.03    | 0.18 ± 0.04 a | 0.27 ± 0.06 a | 0.35 ± 0.06 a |

Values represent mean ± S.D. for 5 rats.
*Significantly different from the 60 kcal-food group (p < 0.05).
was also significantly decreased from 30 kcal-food group (Table 1).

DR and fasting increased the hepatic microsomal proteins without significance. However, the fasting significantly enhanced the total CYP content. As an important subfamily, the CYP2E1 content significantly increased in 15 kcal-food and fasting groups. The demethylation rate of DMN, as a marker of the CYP2E1 activity, was also significantly accelerated in 15 kcal-food and fasting groups (Table 2). In addition, we found that there was good correlation between the amounts of CYP2E1 protein and its activity ($r = 0.94$) (Fig. 2). The hepatic GSH content, which protects liver from hepatotoxic agents, was depleted with the DR and became significant in 15 kcal-food and fasting groups.

In the group where the rats consumed conventional food, the metabolic rate of CHCl$_3$ in vitro was 0.98 nmol/mg protein/min, while that of CCl$_4$ was only 0.11 nmol/mg protein/min, about 1/10 that of CHCl$_3$. The higher metabolic rate of CHCl$_3$ was retained in all food groups. Both the CHCl$_3$ and CCl$_4$ metabolism were enhanced by the decrease of food intake and became significant when the food was restricted to 15 kcal for CHCl$_3$ and when restricted to 30 kcal for CCl$_4$ (Table 2).

Without the administration of CHCl$_3$ and CCl$_4$, one-day DR and fasting did not change either the plasma ALT, AST levels or hepatic GSH content at all. CHCl$_3$ or CCl$_4$ administration at a dose of 3.35 mmol/kg elevated the activities of ALT and AST in all food groups. In general, their activities were higher after CCl$_4$ administration than after CHCl$_3$ administration. The activities of ALT and AST increased with the decrease of food intake and became significantly higher in 30 kcal-food, 15 kcal-food and fasting groups than in 60 kcal-food group. On the other hand, the administration of CHCl$_3$ and CCl$_4$ significantly depleted the hepatic GSH content in all food groups. Although there was no significant difference between two chemicals, the hepatic GSH content tended to be lower after CCl$_4$ administration in the DR groups. When comparing with 60 kcal-food group, CHCl$_3$ significantly decreased the GSH content from 15 kcal-food group and CCl$_4$ significantly decreased its content even from 30 kcal-food group (Table 3).

The toxicokinetics of CCl$_4$ markedly differed from that of CHCl$_3$. Although CHCl$_3$ and CCl$_4$ were administrated at the same dose (3.35 mmol/kg), the blood concentration of CCl$_4$ was much higher than that of CHCl$_3$. As a result, the AUC of CCl$_4$ was as much as twice that of the corresponding CHCl$_3$. On the other hand, The AUC of CHCl$_3$ was markedly small from 15 kcal-food groups in comparison to the 60-kcal group.

![Fig. 2. Correlation between the amount of immunoreactive CYP2E1 protein (arbitrary unit) and CYP2E1 activity (DMN nmol/min/mg protein).]
However, DR only slightly decreased the AUC of CCl₄ (Table 4).

**DISCUSSION**

The effects of fasting on cytochrome enzyme have been researched for several decades. In agreement with earlier studies (Nakajima and Sato 1979; Nakajima et al. 1982; Ueng et al. 1993), we did not find a significant increase in the hepatic microsomal protein. However, the microsomal protein concentration demonstrated an increasing trend with the decrease of food intake ($r = -0.97$). Thus, the slight increase of microsomal protein was not likely to be casual. As a part of the microsomal protein, the CYP content increased significantly by fasting. To our knowledge, this is the first report to find a remarkable increase of CYP due to one-day fasting. An earlier study did not observe any effect even with a 3-day fasting (Nakajima and Sato 1979), and another study found a significant increase of CYP until 3-day of starvation (Imaoka et al. 1990). For the hepatic CYP2E1 content, some studies have shown the significant increase after one-day fasting (Hong et al. 1987; Imaoka et al. 1990). We further demonstrated that CYP2E1 increased significantly not only in the fasted rats but also in the rats consumed 15 kcal of food. Therefore, the complete deprivation of food is not necessary for the significant induction of CYP2E1. It has been...
reported that the DMN demethylase activity is a fundamental activity of CYP2E1 (Yang et al. 1990). This was supported by the present study because there was a good correlation between the amounts of CYP2E1 protein and the demethylation rate of DMN.

After Hong demonstrated that fasting induced a specific form of hepatic P450 in rat liver, CYP2E1 was considered to be an important enzyme induced by fasting (Hong et al. 1987; Imaoka et al. 1990). Because the hepatic CYP2E1 from rats and humans are highly similar, the rat appears to be an excellent model to study the human P450 2E1 expression and function (Wrighton and Stevens 1992). The mechanism of fasting- and DR-induced CYP2E1 is complex because many metabolic and hormonal conditions are involved. For example, hyperinsulinemia was reported to decrease the expression of hepatic CYP2E1 (Peng and Coon 1998). Since insulin decrease can be induced by the lower intake of glucose, glucose starvation may be the common feature for the induction of CYP2E1 (Miller and Yang 1984). This was supported by a Nakajima study that a carbohydrate-free diet increased the CYP2E1 content equivalent to one-day fasting in rats (Nakajima et al. 1982). Although the effects of fasting are not limited to the induction of CYP2E1, the predominant role of CYP2E1 on fasting is beyond doubt. For example, the changes of CYP2B1 content and activity after fasting have produced inconsistent results (Hong et al. 1987; Liu et al. 1993; Brown et al. 1995; Fry et al. 1999). Also, the CYP3A apoprotein caused by fasting alone was weak (Cheesman et al. 1996). To resolve this issue, a well-designed study with many isoforms of CYP is required.

It is well known that hepatic GSH scavenges toxic free radicals and hence protects the liver from hepatotoxic agents. It has been reported that fasting for one or two days caused a remarkable decrease in the hepatic GSH content (Tateishi et al. 1974; Fry et al. 1999). In the present study, the rats in 15 kcal-food group also significantly decreased hepatic GSH content. These results in vitro demonstrated that severe DR for one day not only affected the hepatic metabolism, but also decreased the capacity of hepatic detoxification.

In our previous study, ethanol, a major inducer of CYP2E1, enhanced the hepatotoxicity of CCl₄ and CHCl₃ (Wang et al. 1997). In our other study, fasting potentiated the CHCl₃ toxicity and the extent of potentiation became more obvious with the increasing dose of CHCl₃ (Wang et al. 1995). The dose-effect relationship is one of the most important concepts in toxicology. The present study was designed to observe the dose-effect relationship between the amount of food intake and hepatotoxicity. The administration of CCl₄ or CHCl₃ clearly increased the plasma ALT and AST, the marker enzymes for liver damage, with the decrease of food intake. Food intake restricted to 30 kcal significantly potentiated the hepatotoxicity induced by CCl₄ and CHCl₃. However, CCl₄ was more toxic than CHCl₃ at the same dosage because of the higher plasma ALT and AST levels and lower hepatic GSH content in CCl₄ administration. The reason may be that trichloromethyl radical, a toxic intermediate of CCl₄, is much more reactive and toxic than the presumed ultimate form of CHCl₃, phosgene (Slater 1978). In conclusion, the enzyme induction combined with the decrease of hepatic GSH content after the severe DR and fasting resulted in the augmentation of the hepatotoxicity induced by CCl₄ or CHCl₃.

As two common hepatotoxicants, the metabolic rate was accelerated significantly when food was restricted to 15 kcal (CHCl₃) and even restricted to 30 kcal (CCl₄). This increase can be explained by the remarkable induction of CYP2E1 in the severe DR and fasting. However, CHCl₃ was cleared by hepatic metabolism about 8-10 times faster than that of CCl₄ during DR in the present study and with ethanol administration in our previous study (Wang et al. 1997). It was agreement with the results of in vivo toxicokinetics study where blood concentration (AUC) of CCl₄ was much higher than that of CHCl₃ at the same dosage. These results further supported that CHCl₃ is subject to biotransformation (highly metabolized compound) in the liver, and CCl₄ is resistant to such transformation (poorly metabolized compound) (Sato 1993). We previously
found ethanol treatment predominantly affected the toxicokinetics of high dose of CHCl₃ (1.675 mmol/kg) and it predominately affected that of a low dose of CCl₄ (0.105 mmol/kg) (Wang et al. 1997). Similarly, the severe DR and fasting in the present study significantly decreased AUC of CHCl₃ and had slight effect on AUC of CCl₄ at 3.35 mmol/kg. As described above, CCl₄ is a poorly metabolized compound. Moreover, CCl₄ administered orally enters the liver before reaching the systemic circulation. It is possible that enzyme induced by one-day DR and fasting is not enough to metabolize CCl₄ taken at this high dose to a significant difference from 60 kcal-food group.

In conclusion, when food was restricted to less than half of conventional amount, hepatic metabolism was affected and the hepatotoxicity induced by CCl₄ or CHCl₃ was augmented by, at least in part, CYP2E1 induction and GSH depletion. Although we did not find any effect after the slight DR for one day, the further study is required to observe whether slight DR for long term affects the hepatic metabolism and hepatotoxicity.

References
Table 2. Effect of food restriction on microsomal protein, CYP, CYP2E1, DMN and metabolic rate of CHCl₃ and CCl₄ in vitro.

<table>
<thead>
<tr>
<th>Food intake (kcal)</th>
<th>60</th>
<th>45</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal protein (mg/g liver)</td>
<td>17.5±2.3</td>
<td>18.1±1.2</td>
<td>18.9±1.4</td>
<td>19.6±2.3</td>
<td>20.3±1.5</td>
</tr>
</tbody>
</table>

175 rats in powder food (about 60 kcal) and water

2 weeks

10:00 Five groups: 60, 45, 30, 15 kcal-food and fasting groups

24 hours

10:00 Decapitation (5 × 5 rats)

Liver:

- GSH
- Microsomal protein
- CYP, CYP-2E1, DMN
- Metabolic rates of CHCl₃, CCl₄

Plasma:

- AUC of CHCl₃, CCl₄
- GSH

Table 1. Effect of food restriction on body weight and liver weight of rats.

<table>
<thead>
<tr>
<th>Food intake (kcal)</th>
<th>60 (Control)</th>
<th>45</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>255 ± 4</td>
<td>251 ± 7</td>
<td>246 ± 3</td>
<td>240 ± 1</td>
<td></td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>10.8 ± 0.6</td>
<td>10.2 ± 0.7</td>
<td>9.3 ± 0.9</td>
<td>7.8 ± 0.8</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>LW/BW (%)</td>
<td>4.2 ± 0.3</td>
<td>4.1 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values represent mean ± s.d. for 5 rats.

Table 3. Effect of food restriction on the metabolism and hepatotoxicity of CHCl₃ and CCl₄ in rats.

<table>
<thead>
<tr>
<th>Food intake (Kcal)</th>
<th>45</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHCl₃</td>
<td>80 ±20</td>
<td>83±24</td>
<td>68±24</td>
<td>50±15</td>
</tr>
<tr>
<td>CCl₄</td>
<td>128±26</td>
<td>154±34</td>
<td>186±59</td>
<td>250±95</td>
</tr>
</tbody>
</table>

Values represent mean ± s.d. for 5 rats.

LW/BW, liver weight (g)/body weight (g).

AST (IU/l)

5 rats.

a Significantly different from the 60 kcal food group (controls) (p <0.05).
### Table 4. Effect of food restriction on the areas under the blood concentration-time curve (AUC_{0→24}) of CCl₄ and CHCl₃.

<table>
<thead>
<tr>
<th>Food intake (kcal)</th>
<th>60</th>
<th>45</th>
<th>30</th>
<th>15</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃ (mM×hr)</td>
<td>4.25±0.44</td>
<td>4.01±0.57</td>
<td>3.83±0.46</td>
<td>2.59±0.38 a</td>
<td>2.30±0.29 a</td>
</tr>
<tr>
<td>CCl₄ (mM×hr)</td>
<td>8.54±0.94 b</td>
<td>8.29±1.08 b</td>
<td>7.94±0.90 b</td>
<td>7.67±1.12 b</td>
<td>7.59±1.09 b</td>
</tr>
</tbody>
</table>

Values represent mean ± s.d. for 10 rats.

a Significantly different from the 60 kcal food group (controls) (p <0.05).
b Significantly different from CHCl₃ administration (p <0.05).