Modulations by dietary restriction on antioxidant enzymes and lipid peroxidation in developing mice

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Wu, Aiguo, Xiufa Sun, Fada Wan, and Yugu Liu. Modulations by dietary restriction on antioxidant enzymes and lipid peroxidation in developing mice. J Appl Physiol 94: 947–952, 2003.—The effects of dietary restriction (DR) on the activities of liver superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPX) and the level of lipid peroxidation (LP) in developing mice were investigated in this study. Male and female Kunmin mice were fed a standard rodent diet ad libitum (AL), 80% of AL food intake (20% DR), or 65% of AL food intake (35% DR) for 12 or 24 wk. Both 12 and 24 wk of DR resulted in retarded body weight gain in male and female mice. The activities of SOD, Cat, and GPX and the content of LP in DR male and female mice were not different (P > 0.05) from those in controls after 12 wk of DR. However, the SOD activity was increased at 24 wk in 20% DR (P < 0.05) and 35% DR (P < 0.01) male, but not in DR female, mice. The Cat activity was elevated at 24 wk in both DR male (P < 0.05 for 20% DR, P < 0.01 for 35% DR) and female (P < 0.01) mice with a greater increase in DR female (P < 0.05) than in DR male animals. GPX activity was also increased at 24 wk in DR male (P < 0.01) and female (P < 0.01) mice with a greater elevation in DR females (P < 0.05) than in DR males. Furthermore, LP was decreased at 24 wk in both DR male (P < 0.01) and female (P < 0.01) animals with a greater reduction in DR females (P < 0.01) compared with DR males. These findings indicated that 24 wk, but not 12 wk, of DR led to differential effects on liver SOD, Cat, and GPX activities and LP content in male and female mice during development, suggesting sex-associated modulations of DR on antioxidant systems in developing animals.

IT IS WELL KNOWN THAT reactive oxygen species (ROS) can damage proteins, lipids, and DNA, playing a significant role in numerous diseases, including atherosclerosis, cancer, diabetes, and neurodegenerative disorders (1, 3, 4). ROS also mediates cytotoxicity of many environment chemicals, such as 2,3,7,8-tetrachlorodibeno-p-dioxin (38), arsenic (21), and cadmium (36). The generation of ROS involves normal cellular metabolism (17) and oxidation of a variety of cytotoxic agents such as bleomycin (9). The antioxidant systems, including antioxidants and antioxidant enzymes, can ameliorate the deleterious effects of ROS in vivo and in vitro. Antioxidant enzymes including superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPX) function, by catalyzing the decomposition of oxidants and free radicals. Interventions to increase antioxidant capacity and reduce oxidative damage have been suggested as a potentially useful strategy to prevent or retard the adverse actions of ROS. It has been suggested that dietary restriction (DR) might extend life span, reduce incidence and degree of numerous pathologies, and increase resistance to environmental chemicals by limiting free radical production (10, 25, 30), improving detoxification of free radicals (11, 19, 31), and inducing repair of oxidative damage (29). This hypothesis has been supported by many reports (5–7, 18–20, 25, 26, 29, 31, 36, 40).

It is known that the liver is an important organ for fighting against toxic injury of xenobiotics and endogenous toxins, in which ROS might be involved. Keeping the balance of ROS production and free radical scavenging plays an important role in maintaining the normal function of liver. The majority of findings regarding the beneficial effects of DR on liver antioxidant systems were obtained in aged animals, and the observations might vary in different species of animals. For example, Koizumi et al. (19) reported that long-term DR increased liver Cat activity and decreased lipid peroxidation (LP) in 12- and 24-mo-old female C3B10RF1 mice. The study of Chipalkatti et al. (8) showed that 12-mo-old mice fed one-half of the ad libitum intake showed lower LP levels in the liver and no effect on SOD activity. Ross (33) reported that the specific activity of liver Cat tended to be higher in old restricted rats, but no effects were found in younger rats. Furthermore, the results of Rao et al. (31) showed that DR (40% restriction of energy intake) increased the activities of liver SOD and Cat at 21 and 28 mo of age and GPX at 28 mo of age in male Fischer 344 rats. These studies suggested that the beneficial effects of DR on liver antioxidant systems might be related to sex, age or species of tested animals. However, it remains unknown whether there are different modulations of DR on antioxidant systems in male and female

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developing animals. Therefore, the effects of DR on liver SOD, Cat, and GPX activities and LP level in developing mice were investigated in this study.

MATERIALS AND METHODS

Animals. Male and female Kunmin mice, all 1 mo of age, were housed in individual cages and maintained in environmentally controlled rooms (22 ± 2°C and 50 ± 10% relative humidity) with a 12:12-h light-dark cycle. They were randomly divided into three groups: control, fed ad libitum (AL); 20% DR, fed 80% of AL food intake; 35% DR, fed 65% of AL food intake. Water was available ad libitum. Body weight was determined weekly. Animal care was consistent with the guidelines set by the Laboratory Animal Center of Tongji Medical University. All experimental procedures were approved by the Institutional Research Committee of Tongji Medical University.

Diets. The control and restricted diets were prepared according the compositions as previously described (43) and shown in Table 1. The food consumption in the AL (control) animals was measured daily. The premeasured amount of restricted diets (relative to 80 and 65% of control mice daily consumption) was given daily to the 20% DR and 35% DR animals, respectively. The contents of protein, fat, vitamins, and minerals were adjusted so that intake of these nutrients would be constant in control and restricted mice (Table 1) to avoid malnutrition. Food was changed daily to avoid degradation problem.

Tissue preparation. After 12 wk or 24 wk on control or restricted diet, the mice were anesthetized, and the livers were then removed, immediately frozen in liquid nitrogen, and stored at −70°C until used for the following assays.

Measurement of antioxidant enzyme activities. The activity of SOD was determined by monitoring the inhibition of the autoxidation of pyrogallol (22). One unit of SOD activity was defined as the amount of enzyme required to inhibit pyrogallol autoxidation by 50%. The Cat activity was assayed according to the reported method (2). One unit of Cat activity was defined as the amount of enzyme required to decompose 1 μmol of hydrogen peroxide (H2O2) per minute. The GPX activity was determined by means of a coupled reaction system assay (39). One unit of GPX activity was defined as the amount of enzyme needed to catalyze the oxidation of 1 nmol NADPH per minute.

Measurement of LP. LP level was quantified by measuring thiobarbituric acid-reactive substances production as previously described (12). The values of thiobarbituric acid-reactive substances were expressed as nanomoles of malondialdehyde equivalents per gram of tissue.

Table 1. Composition of diets in each group

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Restriction Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% (Control)</td>
</tr>
<tr>
<td>Protein*</td>
<td>200</td>
</tr>
<tr>
<td>Fat*</td>
<td>50</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>650</td>
</tr>
<tr>
<td>Minerals mix†</td>
<td>40</td>
</tr>
<tr>
<td>Vitamins mix†</td>
<td>20</td>
</tr>
<tr>
<td>Fiber</td>
<td>10</td>
</tr>
<tr>
<td>H2O2</td>
<td>30</td>
</tr>
</tbody>
</table>

Values are given in g/kg. DR, dietary restriction. *The contents of indicated ingredients were adjusted so that intake of these nutrients would be constant. †Prepared as previously described (32).

RESULTS

Food consumption and body weight. The food intake, in grams of food consumed, was 80% in 20% DR group and 65% in 35% DR group relative to control (AL) animals. Body weight gain of DR mice was reduced relative to control except that of females with no difference between 20% DR compared with control group at 12 wk (Fig. 1), suggesting that DR retarded the body weight gain in developing mice. There were no significant differences in food consumption per gram body weight in all groups of male and female mice (data not shown).

SOD activity. The SOD activity in DR male and female mice was not significantly different (P > 0.05) from that in control animals at 12 wk (Fig. 2A). However, the activity of SOD was significantly increased at 24 wk in 20% DR (P < 0.05) and 35% DR (P < 0.01) male, but not in DR female, mice relative to control mice (Fig. 2B).

Cat activity. The Cat activity in DR male and female mice was not significantly different (P > 0.05) from that in control animals at 12 wk (Fig. 3A). However, the Cat activity was elevated at 24 wk in both DR male
suggesting sex-associated modulations of DR on antioxidant systems in developing animals.

The majority of findings regarding the beneficial effects of DR on liver antioxidant systems were obtained in aged animals, and the results may be related to sex and/or species of animals. For example, long-term DR has been reported to increase liver Cat activity and decrease LP in aged female C57B10F1 mice but to have no effect on SOD activity (19). Another report showed that DR increased the activities of liver SOD, Cat, and GPX in aged male Fischer 344 rats (31). In this study we provide evidence for the first time that DR produced different effects on liver antioxidant systems in developing animals. Our results demonstrated that DR significantly increased SOD activity in male mice with no effect on SOD activity in female mice but that it elicited a greater increase in activities of Cat and GPX in female mice \((P < 0.05)\) than in male animals. Furthermore, DR triggered a greater reduction of LP content in female animals \((P < 0.01)\) compared with males. The sex-related effects of DR on antioxidant enzymes and LP in liver of developing animals were not extensively studied; hence the mechanisms underlying DR-induced different modulations on antioxidant systems in animals during development remain to be further investigated.

The DR regimen in this study, including different restriction level of energy intake (0% as control, 20% DR, and 35% DR) with consistent intake of essential nutrients, led to different effects on liver antioxidant systems in male and female mice. However, the sex-related effects of DR on antioxidant enzymes and LP in liver of developing animals were not extensively studied; hence the mechanisms underlying DR-induced different modulations on antioxidant systems in animals during development remain to be further investigated.

**DISCUSSION**

In this study, we investigated the effects of DR on the activities of liver SOD, Cat, and GPX and the level of LP in developing mice. Our results demonstrated that 24 wk, but not 12 wk, of DR led to differential effects on liver SOD, Cat, and GPX activities and LP content in male and female mice during development,
nutrients in all groups, was based on previous evidence (41). The beneficial effects of DR appear to depend on restriction of energy intake with adequate intake of essential nutrients (41). Therefore, optimal nutrient composition and feeding strategies for DR experiments such as levels of restriction, intake of essential nutrients, and term of restriction are very important for designing experiments associated with DR. On the basis of these considerations, we introduced three levels of energy intake (0%, 20%, and 35% DR) with equal intake of fat, protein, vitamins, and minerals to developing mice. Thus our DR regimen (restriction of energy intake without malnutrition) is different from short-term fasting or food deprivation, which may lead to malnutrition, subsequently causing pathological or dysfunctional status in mammalian systems such as reduced detoxification in the liver (14, 23, 37). In this study, we found that DR affects the body weight of both male and female mice in a restriction level-dependent way except that of females with no difference between 20% DR compared with control group at 12 wk (Fig. 1). The reason for these findings is unknown in this study. Furthermore, DR also modulated SOD activity in male mice (Fig. 2B) and Cat activity and LP content in both male and female mice in a restriction level-dependent way (Figs. 3B and 5B).

The reduction of LP observed in the liver of male and female mice may be related to the increase of SOD, Cat, or GPX activity. It is known that SOD converts superoxide anion into H$_2$O$_2$ and O$_2$ (28), whereas Cat and GPX reduce H$_2$O$_2$ to H$_2$O (13, 42), resulting in the detoxification of free radicals. Thus elevation of SOD, Cat, and GPX activities may contribute to the decrease of LP. Another mechanism may be DR-induced reduction of energy expenditure, consequently leading to lowered ROS (30). Reduced LP products suggest decreased formation of fatty acid epoxide and subsequent free radical damage to cellular macromolecules. In addition, DR may lead to reduced mitochondrial oxyradical production and/or increased expression of cytoprotective stress proteins, which may suppress oxyradical production and stabilize cellular homeostasis (25).

SOD, Cat, and GPX are main detoxifying enzymes in cells. SOD appears to be an important enzyme for the prevention of aging and mutation by oxidative stresses and hazardous effects from environmental factors (34). Cat plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (16). GPX also plays important role in protecting mammalian cells against oxidative damage (24). Thus the beneficial effects of DR on these enzymes may promote the capacity of liver to protect against toxic actions of ROS, maintaining normal function. However, the mechanisms underlying elevation of antioxidant enzymes by DR in developing mice need further investigations. It was reported that increase of antioxidant enzyme activities might arise from changes of mRNA (31). It has been described that the expression of these enzymes is regulated by transcriptional con-

![Fig. 4. Effect of DR on liver glutathione peroxidase (GPX) activity in male and female mice. A: 12 wk of DR. B: 24 wk of DR. Values are means ± SE; n = 10 mice in each group. *P < 0.05. **P < 0.01.

Fig. 4. Effect of DR on liver glutathione peroxidase (GPX) activity in male and female mice.](image1)

![Fig. 5. Effect of DR on liver lipid peroxidation level in male and female mice. A: 12 wk of DR. B: 24 wk of DR. MDA, malondialdehyde. Values are means ± SE; n = 10 mice in each group. **P < 0.01.

Fig. 5. Effect of DR on liver lipid peroxidation level in male and female mice.](image2)
control (35), and genetic regulatory elements and promoter sequences for antioxidant enzyme genes have been recognized (27). Furthermore, the evidence of posttranscriptional controls was also presented for antioxidant enzymes (15).

In conclusion, our results indicated that DR (restriction of energy intake with maintenance of essential nutrients) produced differential effects on liver SOD, Cat, and GPX activities and LP level in male and female mice during development, suggesting sex-associated modulations of DR on antioxidant systems in developing animals. These modulations include elevation of SOD activity in male mice and increase of Cat and GPX activities and reduction of LP level in both male and female mice with a stronger effect in female animals compared with males. Because DR is considered to be potentially useful in extending life span of experimental animals, improving outcome of neurodegenerative diseases and producing protections against environmental chemical-induced toxicity, it is mandatory to investigate all possible physiological effects, including those in developing animals. Our findings for the first time provide important insights into the understanding of DR-related physiological implications in modulating antioxidant systems in developing animals and call for further investigations to address the mechanisms underlying DR-induced sex-associated effects on antioxidant systems. Furthermore, more attention should be paid to the potential different influence of DR on antioxidant systems when designing DR-related experiments for basic research, bioassays, and/or toxicity studies by using developing animals; otherwise, DR-induced different effects may lead to variable results in male and female developing animals, resulting in complicated interpretation of data.

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REFERENCES


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