Nutritional Sciences 3(1), May, 2000, pp.11-17
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Reciprocal Effect of DHEA and Dietary Fat on Glutathione Utilizing Detoxifying System in Rat Liver Tissue

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ABSTRACT

This study was intended to examine whether dehydroepiandrosterone (DHEA) and dietary fat level or source could modulate glutathione utilizing detoxifying system activity and the cytosolic NADPH generation in rat liver. Male Sprague-Dawley rats were fed semipurified diet containing either 2% (w/w) corn oil (low level of corn oil diet: 5 cal% of fat), 15% corn oil (high level of corn oil diet: 31 cal% of fat) or 13% sardine oil plus 2% corn oil (high level of fish oil diet: 31 cal% of fat) for 9 weeks. Half of the rats in each diet group were fed a diet supplemented with 0.2% DHEA (w/w). DHEA administration increased plasma total cholesterol level in low corn oil diet-fed rats. The high fish oil diet significantly decreased plasma total cholesterol level compared to the high corn oil diet. Plasma triglyceride level was not significantly changed by DHEA administration and dietary fat level and source. Fasting plasma glucose level was increased by DHEA administration and fish oil diet. Glucose 6-phosphate dehydrogenase activity in liver tissue was significantly reduced by DHEA administration and high fat diet, especially fish oil diet. Malic enzyme activity in liver tissue was significantly increased by DHEA administration. DHEA suppressed the glutathione peroxidase, glutathione reductase and glutathione-s transferase activities in rat liver tissue. The high corn oil diet reduced the activities of these three glutathione-dependent enzymes compared to the low corn oil diet, while fish oil diet elevated the activity of glutathione peroxidase and glutathione reductase compared to corn oil diet. These results suggest that DHEA administration and high level of corn oil diet may suppress the cellular detoxifying system activity through reduction of glutathione utilization, while the fish oil diet did not show these effects.

KEY WORDS: dehydroepiandrosterone, glutathione utilizing detoxifying system, sardine oil, malic enzyme, glucose 6-phosphate dehydrogenase.

INTRODUCTION

Dehydroepiandrosterone (DHEA) treatment decreased body fat and blood lipid level, and prevented or delayed the onset of diabetes, hemolytic anemia, autoimmune disease, and atherosclerosis in several rodent models at a concentration ranging from 0.2 to 1.0% (w/w) in diet.[1-3] Some of these effects are associated with the change of fat metabolism, such as stimulation of lipolysis and/or suppression of lipogenesis.[4-6] It was suggested that the antiobesity and antitumor effects of DHEA were related to the inhibition of glucose 6-phosphate dehydrogenase activity with a concomitant decrease in intracellular levels of ribose 5-phosphate and NADPH, which is a cofactor required for fatty acid synthesis.[7,8]

However, it was reported in some studies that DHEA was hepatocarcinogenic,[9-11] though the mechanism of hepatocarcinogenesis induced by DHEA remains controversial. One hypothesis proposed that DHEA-induced hepatocarcinoma could be caused by an imbalance between generation of H_2O_2 and oxygen radical by peroxisomal β-oxidation of fatty acid and cytochrome P450, and decomposition of H_2O_2 by catalase and other scavenging systems.[12,13] However, there are few studies concerning the effects of DHEA on the glutathione utilizing enzyme, which makes up the major cellular detoxifying system against oxidative stress. Glutathione-utilizing enzymes in liver cytosol, such as glutathione peroxidase, glutathione reductase and glutathione-s transferase, scavenge or deactivate the reactive oxygen species. To improve its efficiency for detoxification, recycling of glutathione is required where NADPH is necessary mainly supplied by either glucose 6-phosphate dehydrogenase or malic enzyme in cytosol. But interestingly, liver cytosolic malic enzyme is induced by DHEA treatment, while glucose 6-phosphate dehydrogenase is inhibited.[14]

Fish oil, being rich in long chain polyunsaturated fatty acids such as eicosapentaenoic acid (20 : 5, n3) and docosahexaenoic acid (22 : 6, n3), inhibits chemical-induced

Accepted : February 2, 2000
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tumorigenesis despite the induction of peroxisomal β-oxidation and lipid peroxidation.[15][16] It was suggested that the antitumorigenic and hypolipidemic effects of fish oil were caused by deactivation of lipogenic enzymes and activation of the glutathione utilizing detoxifying system in liver.[17-20]

Owing to the fact that DHEA is growing in popularity and that fat intake is increasing, it was judged necessary to analyze the combined effects of DHEA and dietary fat on the serum lipid profile and defense system utilizing glutathione. The purpose of this study was to determine whether long-term administration of 0.2% of the low dose of DHEA to rats might affect the NADPH production and glutathione-utilizing detoxifying system in the liver, and whether dietary fat levels and sources could modulate the effects of DHEA.

MATERIALS AND METHODS

1. Materials

Corn oil was obtained from Jeil Jedang Co. (Korea) and sardine oil was donated by Jungyeun Chemical Co. (Korea). DHEA and other chemicals were obtained from the Sigma Chemical Co. (St Louis, MO., USA).

2. Animals and experimental design

Male Sprague-Dawley rats, 8 weeks of age, were purchased from Daehan Experimental Animal Co., and maintained under standard conditions (22-25℃, 50-55% of humidity, 12 hrs light/dark cycle). AIN 76 semipurified diet was provided for all animals to be acclimatized for one week. They were randomly assigned to one of six groups (n = 6-8 per group) and fed different experimental diets (Table 1) for 9 weeks ad libitum: (1) Low level of corn oil diet (LC): 5 cal% of fat, 2% (w/w) of corn oil (2) Low level of corn oil diet containing 0.2% DHEA (LCD) (3) High level of corn oil diet (HC): 31 cal% of fat, 15% (w/w) of corn oil (4) High level of corn oil diet containing 0.2% DHEA (HCD) (5) High level of fish oil (HF) 31 cal% of fat, 13% (w/w) of sardine oil plus 2% (w/w) of corn oil (6) High level of fish oil diet containing 0.2% DHEA (HFD) group. The energy proportions of carbohydrate: protein:fat were 73.5 : 21.6 . 4.9 in the low fat diet and 50.6 : 18.4 : 31.0 in the high fat diet. Experimental diets were prepared every 2 or 3 days and stored at -20℃. Fresh food was supplied to rats every day.

3. Sample preparation

All animals were killed by decapitation after fasting for 18 hrs. Plasma was prepared by centrifuge at 1,000 g for 20 min after collecting blood in heparinized vacutainer (Green Cross Co, Korea) and stored at -70℃ in aliquorts until analysis. Liver was weighed and frozen rapidly in liquid nitrogen for biochemical analysis.

4. Preparation of subcellular fractions

Liver was homogenized in nine volumes of ice-cold 11.5% KCl with 0.2 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM dithiothreitol (DTT). Homogenates were centrifuged at 800 g for 10 min to remove cell debris and nuclei. The supernatant (10% liver homogenate) was collected for the peroxisome rich fraction.

Table 1. Diet composition for experiment (g/kg)

	Low fat		High fat			
	LC	LCD	HC	HCD	HF	HFD
Corn starch	400	400	350	350	350	350
Sucrose	280	280	200	200	200	200
Casein	200	200	200	200	200	200
Corn oil	20	20	150	150	20	20
Sardine oil[1]	-	-	-	-	130	130
α-cellulose	47	47	47	47	47	47
DL-methionine	3	3	3	3	3	3
AIN vitamin mix[2]	10	10	10	10	10	10
AIN mineral mix[3]	40	40	40	40	40	40
DHEA[4]	-	2	-	2	-	2
α-Tocopherol acetate[5]	-	-	-	-	0.044	0.044
Energy density (kcal/100g)	370	370	435	435	435	435
CHO : Prot : Fat (cal%)[6]	73 5 21.6 . 4.9		50.6 18.4 · 31.0			

LC: Low level of corn oil diet; LCD: Low level of corn oil diet containing 0.2% DHEA; HC. High level of corn oil diet; HCD. High level of corn oil diet containing 0.2% DHEA. HF High level of fish oil diet. HFD: High level of fish oil diet containing 0.2% DHEA

1) It contains 7.22% of eicosapentaenoic acid (EPA, 20 5, n3) and 27.0% of docosahexaenoic acid (DHA, 22 6, n3)

2)3) Nutritional Biochemicals. ICN Science Group. Clevland, Ohio

4) Dehydroepiandrosterone (Sigma D-4000)

5) (±)-Tocopherol acetate: 1360 IU/g (Sigma T-3376)

6) The energy proportion of carbohydrate, protein and fat in diet

5. Biochemical analysis

1) Plasma

The levels of plasma protein, albumin, glucose, total cholesterol, HDL-cholesterol and total triglyceride were measured with Technicon autoanalyzer.

2) Liver

Malic enzyme and glucose 6-phosphate dehydrogenase activities in liver homogenate were measured by the methods of Hsu *et al.*[21] and Lohr *et al.*[22] respectively. The activities of glutathione peroxidase, glutathione reductase and glutathione-s transferase in liver homogenate were measured according to Tappel,[23] Carberg *et al.*[24] and Harbig *et al.*,[25] respectively. Protein concentration in liver tissue was assayed by the method of Bradford using bovine serum albumin as a standard.[26]

6. Statistical analysis

Data were expressed as mean ± SD. Statistical differences between groups were determined by ANOVA and Duncan's multiple range test in SAS program.

RESULTS

1. Plasma protein, lipid profile and glucose level

Plasma total protein level was not affected by DHEA administration and dietary fat level and source (Table 2). Plasma albumin level was not affected by dietary fat level and source, but it was significantly increased by DHEA administration in rats fed the low-fat diet. Plasma total cholesterol level was significantly elevated by DHEA administration in rats fed the low corn oil diet, but there was no effect of DHEA administration in rats fed the high corn oil or fish oil diet. The high fish oil diet significantly reduced plasma total cholesterol compared to the high corn oil diet, but it did not affect the plasma HDL-cholesterol level and triglyceride level. The plasma triglyceride level was not affected by DHEA administration and dietary fat level and source. DHEA administration elevated the fasting plasma glucose level in both the high corn oil and fish oil diet. The fish oil diet also elevated the fasting plasma glucose level compared to the corn oil diet (Table 2).

2. Glucose 6-phosphate dehydrogenase and malic enzyme activities

The average glucose 6-phosphate dehydrogenase activity in liver tissue was 17.5 ± 2.7, 7.7 ± 1.2, 2.8 ± 1.0 mmol/min/mg protein in LC, HC and HF group, respectively, and 9.5 ± 2.6, 3.6 ± 1.4, 2.4 ± 0.6 mmol/min/mg protein in LCD, HCD and HFD group, respectively (Fig. 1A). The high fat diet significantly reduced glucose 6-phosphate dehydrogenase activity in liver, whereas the fish oil diet had a stronger inhibitory effect. DHEA administration suppressed glucose 6-phosphate dehydrogenase activity compared to the corresponding control, except in fish oil diet-fed rats.

The average of malic enzyme activity was 6.0 ± 1.4, 7.1 ± 0.8, 4.5 ± 1.7 mmol/min/mg protein in the LC, HC and HF groups, respectively, and 16.2 ± 4.5, 11.6 ± 2.1, 9.9 ± 2.3 mmol/min/mg protein in the LCD, HCD and HFD groups, respectively (Fig. 1B). Malic enzyme activity was significantly increased by DHEA administration but was not affected by dietary fat level and source.

3. The activities of glutathione dependent detoxifying enzymes

The average glutathione peroxidase (GPx) activity was 325.5 ± 61.7, 266.6 ± 39.7, 324.3 ± 28.0 mmol/min/mg protein in the LC, HC and HF groups, respectively, and 254.1 ± 19.2, 214.8 ± 30.8, 239.4 ± 28.6 mmol/min/mg protein in the LCD, HCD and HFD groups, respectively. The average of glutathione reductase (GR) activity was

Table 2. Serum protein, lipid profile and glucose level

Variables	LC(n = 6)	LCD(n = 6)	HC(n = 8)	HCD(n = 8)	HF(n = 8)	HFD(n = 8)
Total protein(g/dl)	7.7 ± 0.9	8 1 ± 0.3	8.2 ± 0.6	7.7 ± 0.6	8.0 ± 0.7	7.7 ± 0.3
Albumin(g/dl)	4.2 ± 0.6$^{b1)}$	4.7 ± 0 4^{a}	4.4 ± 0.3^{ab}	4.4 ± 0.3^{ab}	4.3 ± 0.3^{ab}	4.5 ± 0.3^{ab}
T- Chol(mg/dl)[2)]	106 ± 14 9^{bc}	131 ± 25.3^{a}	120 ± 16.5^{ab}	113 ± 15.8^{ab}	88 ± 11 6^{c}	90 ± 15 3^{c}
HDL-Chol(mg/ml)[3)]	48.8 ± 8.4^{abc}	53.0 ± 10.7^{ab}	49.4 ± 8 63^{abc}	55.4 ± 11.61^{a}	37.5 ± 8.88^{c}	41.1 ± 11.05^{bc}
LDL + VLDL(mg/ml)[4)]	55.0 ± 19 9^{b}	78.2 ± 17 5^{a}	70 4 ± 17.0^{ab}	55.4 ± 23.7^{ab}	50.6 ± 16 2^{b}	49.5 ± 7.3^{b}
Triglyceride(mg/dl)	66.0 ± 28.6^{a}	51 0 ± 33.7^{ab}	41.6 ± 30.3^{ab}	32.8 ± 11.7^{b}	38 8 ± 12.6^{ab}	47.3 ± 17.0^{ab}
Glucose(mg/dl)	291 ± 2.5^{c}	28 ± 12.4^{c}	40 ± 16.4^{c}	60 ± 25.0^{b}	59 ± 11.8^{b}	78 ± 7.5^{a}

Values are mean ± SD. 1) Means with different alphabet are significantly different at $p < 0.05$ by Duncan's multiple range test, 2) Total cholesterol, 3) High density lipoprotein cholesterol, 4) Low density lipoprotein cholesterol + Very low density lipoprotein cholesterol = Total cholesterol-HDL cholesterol level

LC: Low level of corn oil diet, LCD Low level of corn oil diet containing 0.2% DHEA; HC High level of corn oil diet, HCD: High level of corn oil diet containing 0.2% DHEA, HF High level of fish oil diet, HFD: High level of fish oil diet containing 0.2% DHEA

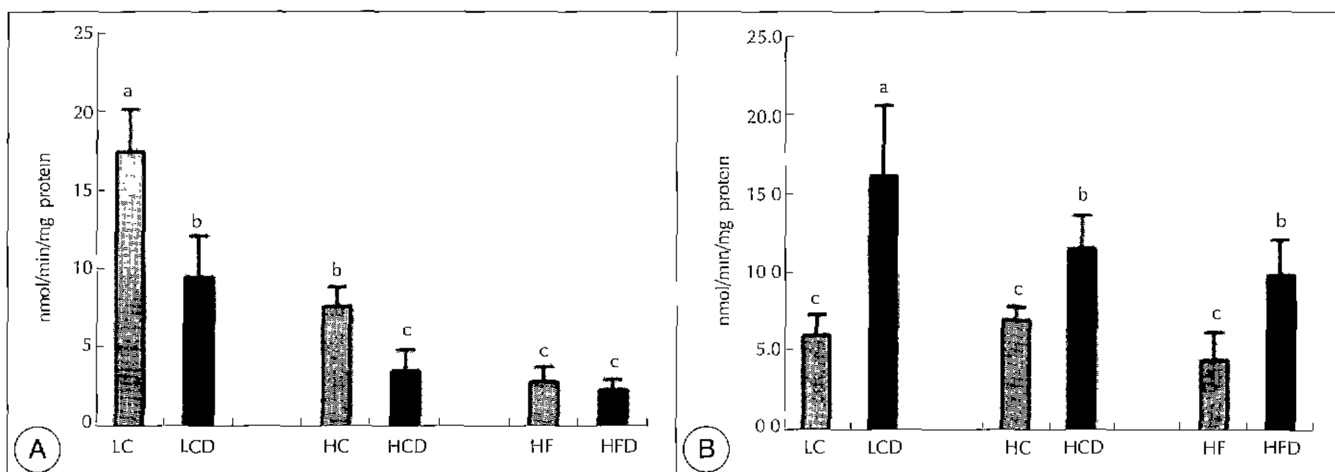

Fig. 1. Glucose 6-phosphate dehydrogenase (A) and malic enzyme (B) activities in rat liver. LC Low level of corn oil diet, LCD. Low level of corn oil diet containing 0.2% DHEA. HC. High level of corn oil diet, HCD High level of corn oil diet containing 0 2% DHEA. HF High level of fish oil diet, HFD High level of fish oil diet containing 0.2% DHEA. Means with different alphabet on the bars are significantly different at $p < 0.05$ by Duncan's multiple range test.

68.3 ± 6.6, 53.5 ± 5.1, 65.9 ± 6.6 mmol/min/mg protein in the LC, HC and HF groups, respectively, and 61. 2 ± 3.5, 48.4 ± 5.6, 54.6 ± 3.5 mmol/min/mg protein in the LCD, HCD and HFD groups, respectively. The average of glutathione-s transferase (GST) activity was 183.2 ± 17.8, 151.2 ± 28.7, 160.8 ± 17.5 mmol/min/mg protein in the LC, HC and HF groups, respectively, and 136.4 ± 11.3, 91.4 ± 19.3, 96.6 ± 10.8 mmol/min/mg protein in the LCD, HCD and HFD groups, respectivly (Fig. 2). All of the activities of GPx, GR and GST in liver tissue were significantly reduced by DHEA administration, regardless of dietary fat level and source. The high corn oil diet reduced the activities of GPx, GR and GST compared to the low corn oil diet. The fish oil diet increased the activities of GPx and GR compared to the corn oil diet.

DISCUSSION

There is much evidence that fish oil reduced the serum cholesterol or triglyceride level through increasing the excretion of cholesterol into bile and fatty acid oxidation, and deactivation of lipogenic enzymes such as malic enzyme and glucose 6-phosphate dehydrogenase.[17-20] It was reported that the hypolipidemic effect of fish oil was accompanied by a significant reduction of plasma insulin level.[20,27] Chiang and Tsai[18] suggested that n-3 polyunsaturated fatty acids might play an important role in the regulation of glucose and lipid metabolism. The results of that study showed that fish oil supplementation to Wistar rats decreased plasma lactate, free fatty acid and cholesterol levels, and in addition, it reduced glucose 6-phosphatase and glucose 6-phosphate dehydrogenase activities and improved glucose tolerance ability. On the other hand, it is known that DHEA alters carbohydrate and lipid metabolism,[17,28] but the effect of DHEA on serum lipid profile is controversial. In some studies, DHEA reduced serum triglyceride or cholesterol,[4,16,29] while in the others, DHEA did not change the serum lipid profile or rather increased serum cholesterol level.[2,6,19,30] This discrepancy might be relate to the difference in DHEA level, dietary fat level or source, animal gender or experimental duration, etc. Nevertheless, the interaction of DHEA with dietary fat on the serum lipid profile has not yet been sufficiently studied. In the present study, the plasma cholesterol level would be more sensitive to dietary fat source rather than DHEA administration because the fish oil diet reduced the plasma total cholesterol level compared to corn oil diet, while DHEA administration did not change the plasma total cholesterol level in high fat diet-fed rats (Table 2). The fact that there was no difference in plasma triglyceride level between control and DHEA-administered rats in this study might be a result of low level of DHEA. The level of 0.2% of DHEA might be too low to have an effect on the serum triglyceride level. In our previous study, the serum triglyceride level was significantly reduced by 1% DHEA administration to rats for 10 days.[16]

In the present study, the fasting plasma glucose level was increased in high fish oil diet-fed rats, and DHEA administration with high fat diet-corn oil and fish oil significantly increased the plasma glucose level (Table 2). These results could be caused by marked increases in peroxismal fatty acid oxidation in liver tissue by both fish oil and DHEA.[16] It is known that fatty acid oxidation is

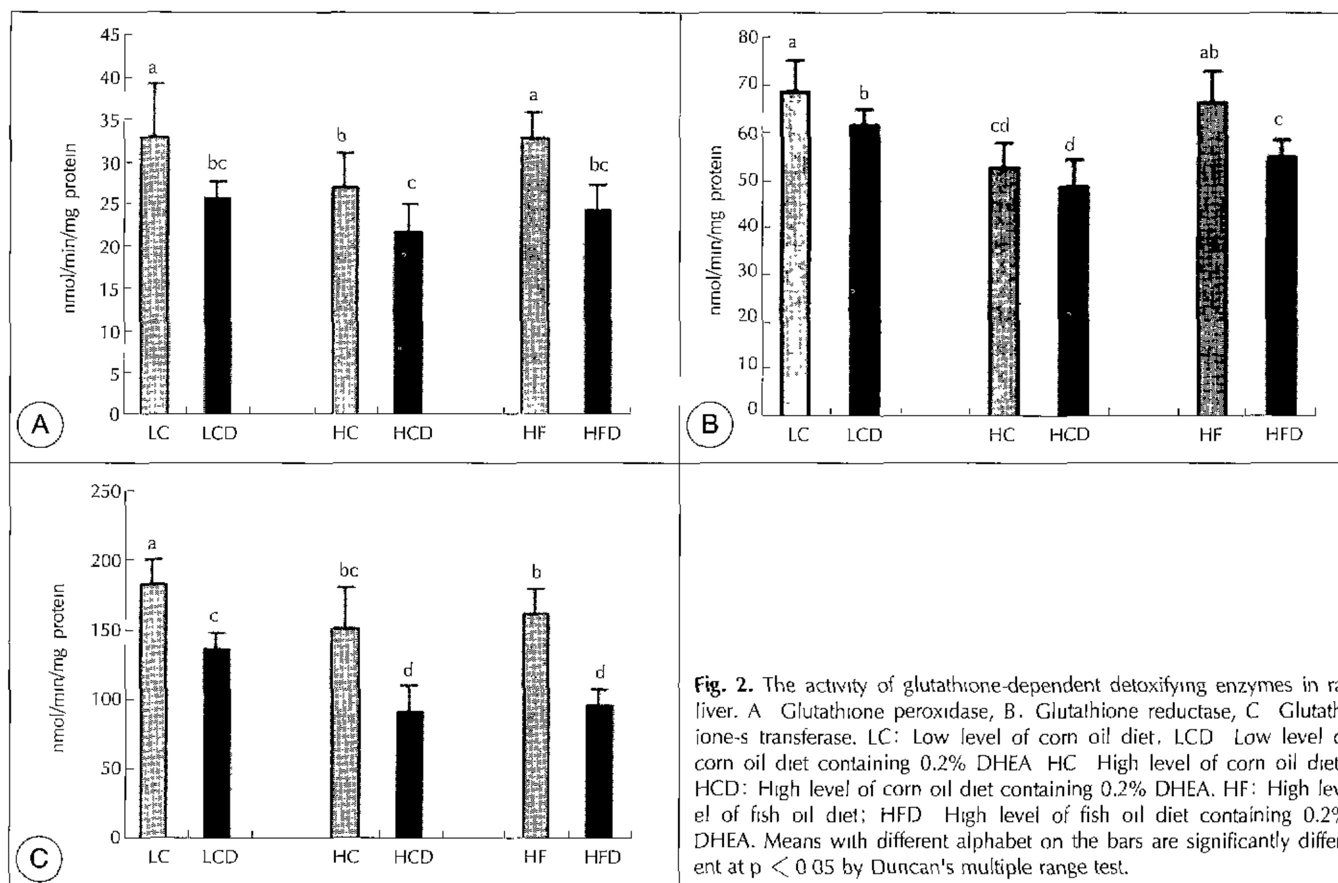

Fig. 2. The activity of glutathione-dependent detoxifying enzymes in rat liver. A Glutathione peroxidase, B. Glutathione reductase, C Glutathione-s transferase. LC: Low level of corn oil diet, LCD Low level of corn oil diet containing 0.2% DHEA HC High level of corn oil diet. HCD: High level of corn oil diet containing 0.2% DHEA. HF: High level of fish oil diet; HFD High level of fish oil diet containing 0.2% DHEA. Means with different alphabet on the bars are significantly different at $p < 0.05$ by Duncan's multiple range test.

essential for maintaining maximal rates of gluconeogenesis, especially from substrates dependent on pyruvate carboxylation.[31,32] The increase of NADH/NAD + ratio caused by the increase in free fatty acid oxidation could decrease the metabolic flux through pyruvate dehydrogenase and increase the metabolic flux through pyruvate carboxylase.[31] Moreover, DHEA administration could produce considerable amounts of pyruvates via the activation of malic enzyme in liver tissue. Mayer *et al.*[32] also suggested that DHEA treatment shifted the carbohydrate metabolism toward energy loss via decreased glucose consumption and increased glucose output. The basis for this contention was that the activities of all the rate-limiting enzymes of glucose metabolism, such as glucose 6-phosphate dehydrogenase, hexokinase, pyruvate kinase, fructose 1,6-bisphosphatase were markedly reduced and glucose 6-phosphatase activity was increased by DHEA administration. The inhibition of glycolysis and activation of glucose output by DHEA enforced the blood glucose level.

The inhibition of glucose 6-phosphate dehydrogenase activity in liver tissue by DHEA administration in this study was consistent with several other studies.[5,33,34] It has been postulated that the decreased availability of reducing equivalents such as NADPH by DHEA could result in a decreased rate of fatty acid elongation.[17] However, malic enzyme, another NADPH-generating enzyme, has been shown to be activated in rat liver treated with DHEA as well as with some other hypolipidemic drugs.[6,14,35] Ayala *et al.*[36] suggested the increase in malic enzyme activity could be a general nonspecific response of the liver to pharmacological agents. The decrease of cytosolic NADPH by inhibition of hepatic glucose 6-phosphate dehydrogenase activity by DHEA administration could be compensated by the increase of malic enzyme activity (Fig. 1). Actually, no change in either the mitochondrial or cytosolic $NADP^+$/NADPH ratio by DHEA administration was observed in some studies.[37,38]

The activities of GPx and GST are directly related to the cellular radical scavenging capacity. In addition, the concomitant activity change of GR activity would regulate the detoxifying capability by modulating the NADPH-dependent glutathione redox cycling. The effects of DHEA administration on the glutathione utilizing system have been controversial. DHEA decreased the activities of GPx and GST in rat liver and increased GR activity in some studies,[9,39] while the activities of GR and GST were increased in DHEA-treated rats in some other studies.[31,40] In our research, we found that DHEA has a strong in-

hibitory effect on GPx, GR and GST activities. The DHEA effects on these enzyme activities were predominant over the dietary fat effects (Fig. 2). The concomitant decreases in the activity of GPx and GR would slow the glutathione redox cycling. And the decrease in GST activity would weaken the cellular detoxifying potential.

These deactivations of the glutathione-dependent enzymes by DHEA would be caused by oxygen radicals generated from peroxisomal proliferation or by down regulation of these enzymes. But the effect of DHEA on oxidative stress has been disputed with respect to the induction of peroxisomal proliferation and cytochrome P 450, as well as its antioxidant activity.[41-44] Further study is needed to define the mechanism. The fish oil-fed rats were shown to have the increased GPx and GR activity, probably stimulated by the marked increase of the lipid peroxide that was shown in several studies.[19,45] GST activity, which was reported to have increased in the fish oil-fed rats,[46-48] was not shown to be increased in this study (Fig. 2).

Our results can be summarized as follows. Plasma cholesterol level was reduced by the fish oil diet, and fasting plasma glucose level was enhanced by DHEA administration only in high fat diet-fed rats. Glucose 6-phosphate dehydrogenase activity was sensitively inhibited by DHEA administration and a high fat diet, especially a fish oil diet, while malic enzyme activity was readily induced by DHEA administration. The activity of glutathione-dependent enzymes including GPx, GR and GST decreased significantly in the DHEA-administered group and it was lower in the high level corn oil diet group than in the low level corn oil diet group, however there was absense of this effect in the fish oil diet group.

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