Effect of Dietary Corticosterone and Vitamin E on Growth and Oxidative Stress in Broiler Chickens

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Abstract The present experiment was conducted to study the effects of dietary corticosterone (CTC) and vitamin E on growth and oxidative stress in broiler chickens. Chicks (Cobb strain) were divided into 3 (vitamin E) × 2 (CTC) blocks. Vitamin E was mixed with basal diet at levels of 0 (E 0), 500 (E 500) and 5,000 (E 5000) mg DL-α-tocopheryl acetate/kg. CTC was mixed with basal or vitamin E diets at a level of 20 mg/kg. The body weight gain was significantly lower when the birds were treated with CTC. However, high dose vitamin E (5,000 mg/kg) reduced the CTC effect. Thus, the feed conversion ratio was higher in the CTC group and lower when vitamin E was given. CTC increased abdominal fat content and decreased adrenal glands weight, and vitamin E tended to reduce these effects. Thiobarbituric acid reactive substance (TBARS) in the liver was elevated by CTC, and it was significantly reduced by high dose vitamin E. The plasma cholesterol concentration was increased by CTC, and vitamin E reduced the effect. The plasma CTC concentration tended to be increased by CTC and this was further unexpectedly increased by vitamin E. In conclusion, the results indicate that CTC induces oxidative stress, hypercholesterolemia and growth inhibition, and these effects of CTC are minimized by vitamin E.

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Domestic animals are often subjected to different type of stresses such as restraint, fasting, transport, and exposure to high or low environmental temperature. During stress, the hypothalamus-pituitary-adrenal axis is activated and glucocorticoid hormone is released from the adrenal cortex[13]. Glucocorticoid is essential for maintaining life and normal growth. However, excessive glucocorticoid causes growth inhibition, muscle proteolysis, fat deposition[8] and hypercholesterolemia[19]. It has been also reported that stress increases formation of free radicals, initiating lipid peroxidation in tissues followed by abnormal lipid metabolism[9]. Fatty acids components of membrane lipids are especially sensitive to hydroxyl radicals and they are transformed in lipid peroxides with the subsequent disruption of some membrane-associated processes[21]. It is also well known that oxidative damage of proteins can result in reduced biological function and enhanced susceptibility to proteolysis[18]. Thus, free radicals trigger the metabolic disorder, cell death[16] and growth retardation. However, little attention has been given to the point that free radical is formed when animals are exposed to stress. There are a number of studies showing the relation between glucocorticoid status and lipid peroxidation *in vitro*[2,17]. However, there is little study investigating the whole-body effect of glucocorticoids on the development of free radicals in animals.

It has been suggested that antioxidant administration reduces the physiological response to stress in animals[1]. The organisms have enzymatic and non enzymatic defenses against free radical and vitamin E is a hydrophobic antioxidant playing major roles in non enzymatic defenses[4]. It acts as a scavenger for free radicals and a terminator of lipid peroxidation[3].

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Under stressful condition, requirement of antioxidants such as vitamin E[15] and vitamin C[20] are thought to be increased to protect tissue from lipid peroxidation and to serve as non-specific reduction system from free radicals injury[16].

For reasons mentioned above, we have speculated that glucocorticoid enhances formation of free radicals and thus the effect of glucocorticoid might be minimized by vitamin E as a radical scavenger. Therefore, in the present study, effects of dietary corticosterone and large amount of dietary vitamin E on growth, plasma cholesterol, plasma CTC and hepatic TBARS used as an index of lipid peroxidation[1] were examined using corticosterone-administered chickens.

Materials and Methods

Animals and schedule

One-day-old male broiler chickens of Cobb strain were supplied by a local commercial hatchery (Kumiai Hina Center, Kajiki, Kagoshima-Prefecture, Japan). They were housed in an electrically-heated battery brooder and were provided with water and a commercial starter diet (21% CP ; Minami Nihon Kumiai Shiryo Kabusiki Kaisha, Kagoshima-City, Japan) *ad libitum* for the first 7 days. On day 7, thirty-six birds of similar body weight (about 150 g) were selected, grouped and housed in wire-bottomed aluminum cages (49 cm height × 40 cm width × 67 cm depth), and six replications were made per treatment. The experiments were conducted in temperature-controlled rooms with 14 h light-10 h dark cycle. The temperature of the room was 25℃, and relative humidity was maintained at 50–70% throughout the experiment.

Experimental diets and feeding

The composition of a basal diet (3,200 kcal ME/kg and 23% crude protein) is shown in Table 1. The basal diet was fed *ad libitum* during the prefeeding period (from 7 to 9 days of age). Thereafter, chicks (153 g±9) were divided into CTC− and CTC+ groups, and each group was further divided into E 0 (n=6), E 500 (n=6) and E 5000 (n=6) blocks. E 500 and E 5000 blocks received the basal diets replaced corn with DL-α-tocopheryl acetate. E 500 and E 5000 diets contained 500 and 5,000 mg vitamin E/kg, respectively. DL-α-tocopheryl acetate was purchased from Nakalai Tesque (Kyoto, Japan). CTC was mixed with basal or vitamin E diets at level of 20 mg/kg. CTC was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The birds were treated with vitamin E and CTC from 9 to 15 days of age and from 11 to 15 days of age, respectively. Body weights and feed intake were recorded daily. At the end of the experimental period, all the birds were sacrificed by decapitation. Blood samples were collected into heparinizedtest tubes, quickly centrifuged at 5,900 × g for 10 min at 4℃ to separate plasma, and stored at −20℃ until analysis. And the birds were dissected to remove pectoral superficial muscle, abdominal fat, liver and adrenal glands. Pectoral muscles weights were used as measures of skeletal muscle growth because pectoral muscles can be easily dissected and their lipid contents are lower than those of the thigh muscles. The rates of growth of breast muscles are similar to those of thigh muscles

Table 1. Composition of basal diet

Ingredients	%
Ground yellow corn	71.66
Dehydrated alfalfa meal	3.77
Purified soybean proteie	19.60
Mineral mixture*	3.31
Vitamin mixturn†	0.26
L-Lysine	0.28
DL-Methionine	0.32
Soybean oil	0.80
Analysis	
Crude protein, %	23
ME	3,200 kcal/kg

* Contained (/kg) : $CaCO_3$ 210 g, $CaHPO_4$ 660 g, NaCl 113 g, $MnSO_4 \cdot 4\text{–}5\,H_2O$ 6.600 g, $ZnSO_4 \cdot 7\,H_2O$ 4,000 g, $FeSO_4$ 6.145 g, $CuSO_4 \cdot 7\,H_2O$ 233 mg, $NaIO_3$ 16 mg, H_2SeO_3 6 mg.

† Contained (/kg) : retinol 1,750,000 IU, cholecalciferol 200,000 IU, riboflavin 2.5 g, thiamine 1 g, pyridoxine 0.5 g, cyanocobalamin 10 mg, pantothenic acid 4 g, nicotinic acid 10 g, menadione 250 mg, folic acid 200 mg, choline chloride 300 mg, biotin 25 mg, DL-a-tocopheryl acetate 2.5 g, sucrose 978 g.

between 2 and 4 weeks of age[12].

Chemical analyses

Plasma cholesterol concentration : Plasma concentration of cholesterol was measured by an enzymatic cholesterol assay using a commercial kit, Cholesterol E-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Lipid peroxidation : Lipid peroxidation in the liver was assessed as the concentration of TBARS by a method of Ohkawa *et al.*[14]. TBARS, in particular, malondialdehyde (MDA), are products of the oxidative degradation of polyunsaturated fatty acids, and thus used as an index of oxidative stress.

Plasma CTC concentration : Plasma CTC concentration was measured by a radioimmunoassay using a commercial kit (ICN Biomedicals, Inc., California, USA).

Statistical analyses

Data were analyzed by two-way analysis of variance (ANOVA) using the General Liner Model procedure of the statistical analysis system software package (Release 6.09, SAS Institute Inc., Cary, NC, USA) with Duncan's multiple-range test. A P value <0.05 was considered to be statistically significant.

Results

The body weight gain, feed intake and feed conversion ratio during 4 days from 11 to 15 days of age are shown in Fig. 1. The body weight gain was markedly decreased when the birds were treated with CTC and this was statistically significant when analyzed by ANOVA. But there was no effect of CTC when high dose vitamin E was given. When the results were expressed as percentage of CTC− group, they were 61 ±10% (E0), 57±19% (E500), 88±12% (E5000), and it was clearly shown that high dose vitamin E significantly minimized the CTC effect. The feed intake was not changed. The feed conversion ratio was significantly higher in the CTC+ group and the CTC effect was reduced by high dose vitamin E. ANOVA showed that the effect of CTC and interaction of CTC and vitamin E on feed conversion ratio were significant.

The relative weights of organs are shown in Fig. 2. The relative weight of pectoral superficial muscle was

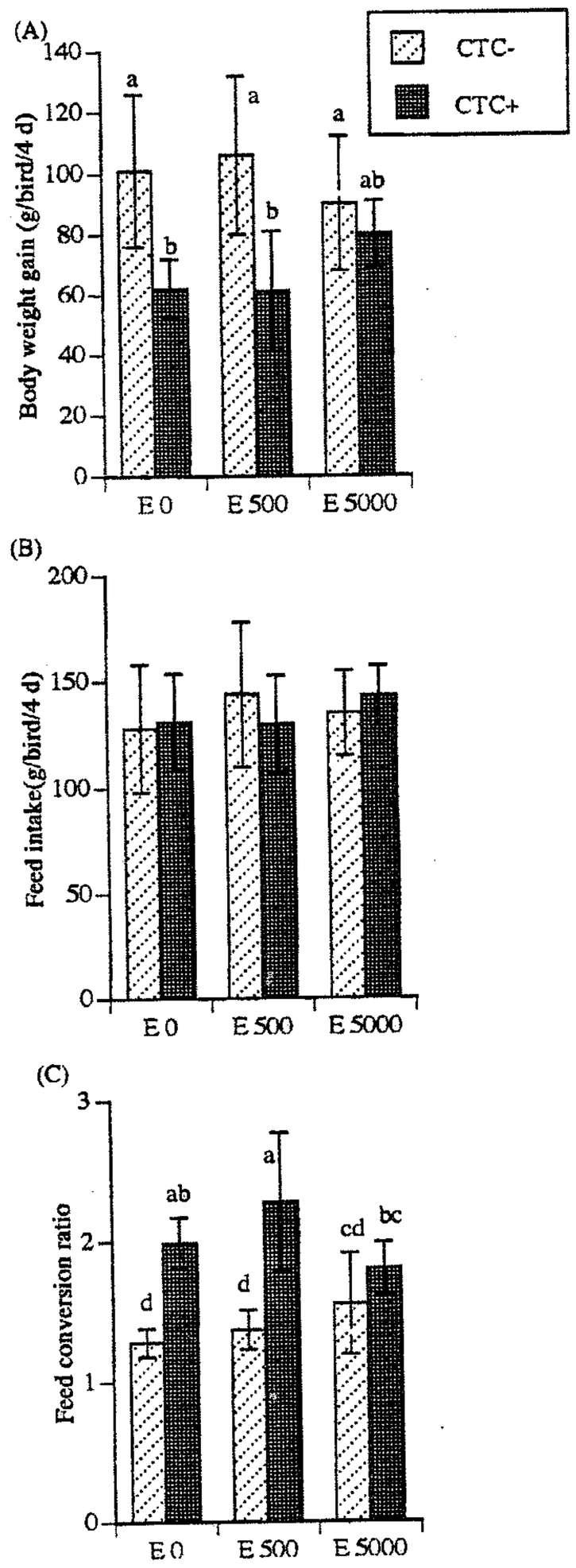

Fig. 1. Effect of corticosterone (CTC) and vitamin E on body weight gain (A), feed intake (B) and feed conversion ratio (C) in male broiler chickens. CTC was included in the diet at the level of 20 mg/kg. E 500 and E 5000 diets contain 500 mg and 5000 mg α-tocopheryl acetate/kg, respectively. Values are expressed as means ±SD. Means bearing different letters are significantly different ($P<0.05$).

ANOVA

	Body weight gain	Feed intake	Feed conversion ratio
CTC	<0.0001	NS	<0.01
Vitamin E	NS	NS	NS
CTC×Vitamin E	NS	NS	<0.05

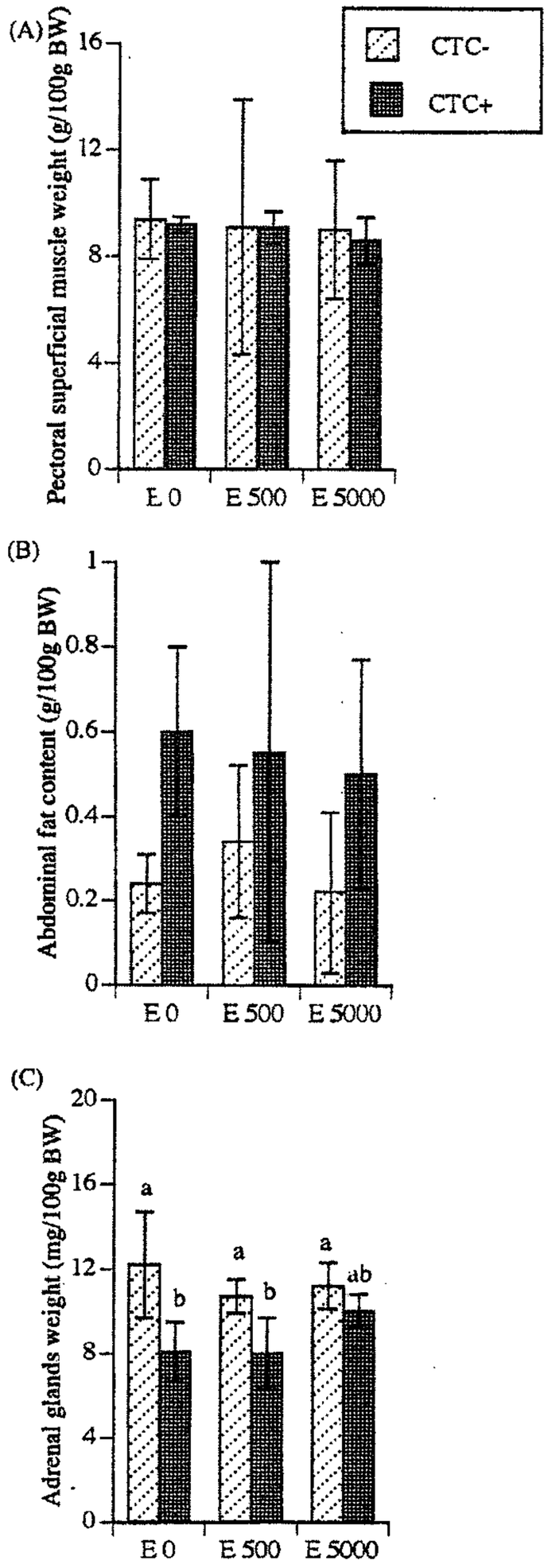

Fig. 2. Effect of corticosterone (CTC) and vitamin E on pectoral superficial muscle weight (A), abdominal fat content (B) and adrenal glands weight (C) in male broiler chickens. CTC was included in the diet at the level of 20 mg/kg. E 500 and E 5000 diets contain 500 mg and 5,000 mg α-tocopheryl acetate/kg, respectively. Values are expressed as means ±SD. Means bearing different letters are significantly different ($P<0.05$).

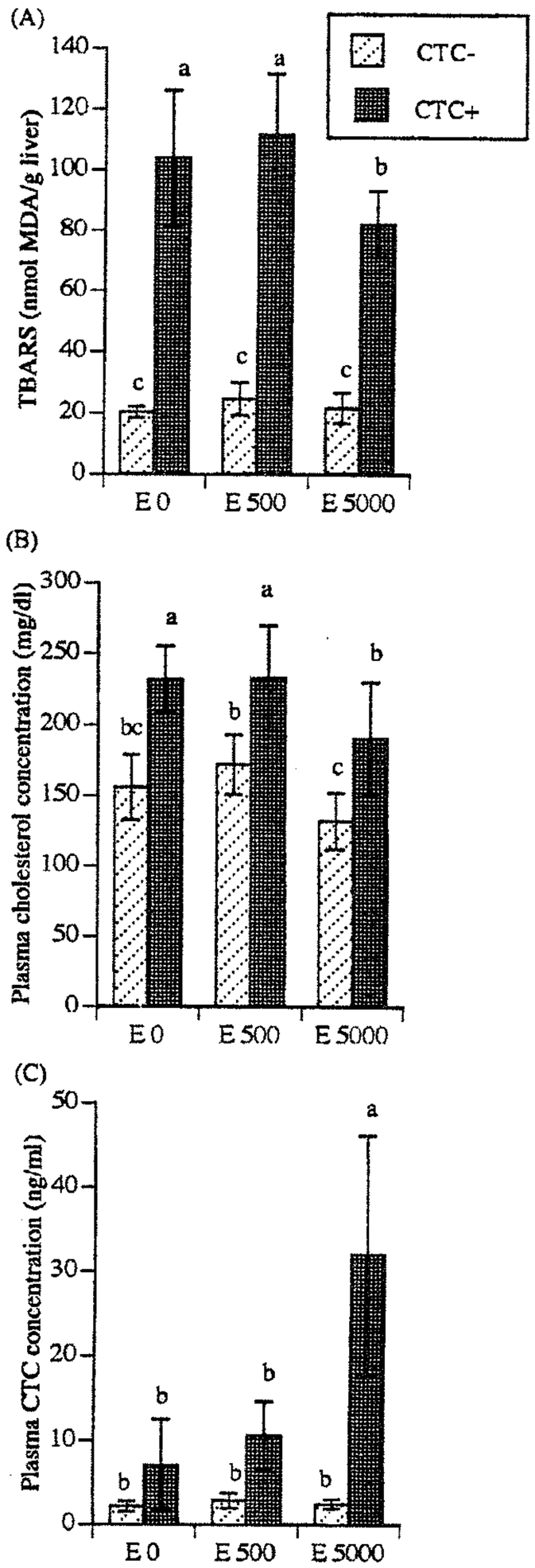

Fig. 3. Effect of corticosterone (CTC) and vitamin E on hepatic TBARS (A), plasma cholesterol concentration (B) and plasma corticosterone concentration (C) in male broiler chickens. CTC was included in the diet at the level of 20 mg/kg. E 500 and E 5000 diets contain 500 mg and 5000 mg α-tocopheryl acetate/kg, respectively. Values are expressed as mean ±SD. Means bearing different letters are significantly different ($P<0.05$).

ANOVA

	Pectoral muscle	Abdominal fat	Adrenal glands
CTC	NS	<0.01	<0.001
Vitamin E	NS	NS	NS
CTC×Vitamin E	NS	NS	NS

ANOVA

	TBARS	Cholesterol	CTC
CTC	<0.01	<0.0001	<0.0001
Vitamin E	<0.05	<0.01	<0.001
CTC×Vitamin E	NS	NS	<0.01

not significantly different from the CTC− (Fig. 2-A). However, absolute weight of the muscle was significantly decreased by dietary CTC (data is not shown). There was no effect of vitamin E on the muscle weight. ANOVA showed that the effect of CTC on abdominal fat content was significant, and vitamin E tended to reduce the effect (Fig. 2-B). Adrenal glands weight was decreased by CTC and the effect tended to be reduced by high dose vitamin E (Fig. 2-C).

As expected, TBARS in the liver was markedly elevated by CTC treatments as shown in Fig. 3-A. The increment in TBARS due to CTC was significantly reduced by high dose vitamin E. The ANOVA clearly showed that feeding vitamin E significantly reduced CTC-induced TBARS increment. It was also shown by ANOVA that plasma cholesterol concentration was significantly increased by CTC, and high dose vitamin E reduced the effects. (Fig. 3-B). Plasma concentration of CTC tended to be increased by CTC and this was unexpectedly further increased by vitamin E (Fig. 3-C). ANOVA showed that the effects of CTC and vitamin E and the interaction of CTC and vitamin E were all significant.

Discussion

We investigated the effects of corticosterone and vitamin E on growth inhibition and lipid peroxidation in chickens using hepatic TBARS as an index of oxidative stress. TBARS was increased when chickens were treated with CTC and it was reduced when large amount of vitamin E was given. This indicates that glucocorticoid induced free radicals production in the liver. CTC also caused hepatic hypertrophy (data is not shown). ANOVA showed that relative liver weight was significantly increased by CTC. These results are consistent with our previous study using rats[15].

It has been reported that the growth of broiler chickens is impaired in a dose-depended manner by dietary corticosterone[8]. In the present experiment, body weight gain was decreased to about 60% of the CTC−. However, this was not caused by the reduction of feed intake. Growth inhibition due to CTC has been mainly explained by enhanced muscle proteolysis[7]. Skeletal muscle proteins may be damaged by oxidative stress as reported by Hunt *et al.*[10]. The growth inhibition due to CTC observed in the present experiment might partly be caused by oxidative stress since high dose vitamin E reduced the growth inhibition. An increment of feed conversion ratio due to CTC was also minimized by high dose vitamin E as was shown by ANOVA. Requirement of vitamin E as an antioxidant is thought to be increased to protect tissue from lipid peroxidation in CTC+ group. Thus, high dose vitamin E may play an important role when animals are exposed to severe stress. Indeed our previous study showed that the increased lipid peroxidation caused by CTC treatment is the result of a reduction in the activities of antioxidative enzymes, and the vitamin E effect is derived from the restoration of the antioxidative enzymes[15].

Despite the growth inhibition and decreases in the muscle weight, abdominal fat content was increased by CTC. It is also well known that glucocorticoids cause hypercholesterolemia[19]. Recent studies show that hypercholesterolemia is accompanied by increases in lipid peroxidation[5,11]. In the present experiment, CTC significantly increased plasma cholesterol concentration and hepatic TBARS. And vitamin E reduced the effects of CTC on both plasma cholesterol and hepatic TBARS. This is consistent with the results of Watkins *et al.*[22] who reported that γ-tocotrienol and α-tocopherol reduced plasma cholesterol and plasma TBARS in hypercholesterolemic rats. These results indicate that CTC-induced abnormalities in lipid metabolism are caused by oxidative stress, and thus vitamin E normalizes these effects.

Chronic corticosteroid administration or stimulation of the adrenal glands results in slower growth in young birds[6]. As expected, adrenal glands weight were significantly decreased by CTC in the present experiment, and the adrenals weight tended to be increased by vitamin E.

Contrary to the expectation, increase in plasma CTC concentration due to CTC treatment was further increased by dietary vitamin E and ANOVA showed that the interaction of CTC and vitamin E was significant. This is very interesting, but we have no data to explain this phenomenon at the present. Vitamin E

and CTC might share the metabolic system such as glucuronide conjugation system in the liver and they might be competitively metabolized by the system. Consequently, plasma CTC concentration might be further increased by high dose vitamin E.

In conclusion, the present study indicates that CTC induces free radicals formation and oxidative stress followed by hypercholesterolemia and growth inhibition, and these effects of CTC are minimized by vitamin E.

References

1) Armario A, Campmany L, Borras M, Hidalgo J. Vitamin E-supplemented diets reduce lipid peroxidation but do not alter either pituitary-adrenal, glucose, and lactate responses to immobilization stress or gastric ulceration. *Free Radical Research Communications,* 9 : 113--118. 1990.
2) Behl C, Lezoualc'h F, Trapp T, Widmann M, Skutella T, Holsboer F. Glucocorticoids enhance oxidative stress-induced cell death in hippocampal neurons *in vitro. Endocrinology,* 138 : 101--106. 1997.
3) Bjorneboe A, Bjorneboe GE, Drevon CA. Absorption, transport and distribution of vitamin E. *The Journal of Nutrition,* 120 : 233–242. 1990.
4) Del Maestro RF. An approach to free radicals in medicine and biology. *Acta Physiologica Scandinavica. Supplementum,* 492 : 153–168. 1980.
5) Eddy AA. Interstitial fibrosis in hypercholesterolemic rats : role of oxidation, matrix synthesis, and proteolytic cascades. *Kidney International,* 53 : 1182. 1998.
6) Freeman BM, Manning AC. The response of the immature fowl to multiple injections of adrenocorticotrophic hormone. *British Poultry Science,* 16 : 121–129. 1975.
7) Goldberg AL, Tischler M, DeMartino G, Griffin G. Hormonal regulation of protein degradation and synthesis in skeletal muscle. *Federation Proceeding,* 39 : 31–36. 1980.
8) Hayashi K, Nagai A, Ohtska A, Tomita Y. Effects of dietary corticosterone and trilostane on growth and skeletal muscle protein turnover in broiler cockerels. *British Poultry Science,* 35 : 789–798. 1994.
9) Hidalgo J, Campmany L, Borras M, Garvey JS, Armario A. Metallothionein response to stress in rats : role in free radical scavenging. *American Journal of Physiology,* 255 : E518–E524. 1988.
10) Hunt J, Dean RT, Wolff SP. Hydroxyl radical production and autoxidative glycosylation. *Biochemical Journal,* 256 : 205–212. 1988.
11) Inoue N, Ohara Y, Fukai T, Harrison DG, Nishida K. Probucol improves endothelial-dependent relaxation and decreases vascular superoxide production in cholesterol-fed rabbits. *American Journal of the Medical Sciences,* 315 : 242–247. 1998.
12) Kang CW, Sunde ML, Swick RW. Growth and protein turnover in the skeletal muscles of broiler chicks. *Poultry Science,* 64 : 370–379. 1985.
13) Klemcke HG, Nienaber JA, Hahn GL. Plasma adrenocorticotropic hormone and cortisol in pigs : Effects of time of day on basal and stressor-altered concentrations. *Proceeding Society for Experimental Biology and Medicine,* 190 : 42–53. 1989.
14) Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* 95 : 351–358. 1979.
15) Ohtsuka A, Ohtani T, Horiguchi H, Kojima H, Hayashi K. Vitamin E reduces glucocorticoid-induced growth inhibition and lipid peroxidation in rats. *Journal of Nutritional Science and Vitaminology,* 44 : 237–247. 1998.
16) Okada S. Iron-induced tissue damage and cancer : the role of reactive oxygen species-free radicals. *Pathology International,* 46 : 311–332. 1996.
17) Slater AF, Nobel CS, Maellaro E, Bustamante J, Kimland M, Orrenius S. Nitrone spin traps and a nitroxide antioxidant inhibit a common pathway of thymocyte apoptosis. *Biochemical Journal,* 15 : 771–778. 1995.
18) Stadtman ER, Oliver CN. Metal-catalyzed oxidation of proteins. Physiological consequences. *The Journal of Biological Chemistry,* 266 : 2005–2008. 1991.
19) Staels B, van Tol A, Chan L, Verhoeven G, Auwerx J. Variable effects of different corticosteroids on plasma lipids, apolipoproteins, and hepatic apolipoprotein mRNA levels in rats. *Arteriosclerosis Thrombosis,* 11 : 760–769. 1991.
20) Takahashi K, Akiba Y, Horiguchi M. Sex-related differences of hepatic microsomal mixed function oxidase system and antibody production in broilers implanted with corticosterone and/or fed ascorbic acid. *Comparative Biochemistry Physiology,* 105 : 159 –164. 1993.
21) Tappel AL. Lipid peroxidation damage to cell components. *Federation Proceeding,* 32 : 1870–1874. 1973.
22) Watkins T, Lenz P, Gapor A, Struck M, Tomeo A, Bierenbaum M. γ-Tocotrienol as a hypocholesterolemic and antioxidant agent in rats fed atherogenic diets. *Lipids,* 28 : 1113–1118. 1993.