Involvement of lipoic acid in plasma metabolites, hepatic oxygen consumption, and metabolic response to a β-agonist in broiler chickens

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The present study was conducted to determine the role of α-lipoic acid (LA) in plasma metabolites, hepatic O_2 consumption, and β-adrenergic response in broilers. In Expt 1, 12-d-old female broiler chicks were divided into three dietary groups and fed on diets with or without LA (5 or 50 mg/kg) until 4 or 6 weeks of age, as a 2×3 factorial arrangement. The dietary LA had no effect on growth rates (body weight, abdominal fat, breast muscle, and liver). The higher level of LA increased plasma non-esterified fatty acid and decreased plasma triacylglycerol concentrations only at 6 weeks of age. A 42 % increase in hepatic respiration was observed in the 4-week-old chickens given 50 mg LA/kg diet. In Expt 2, 3-d-old female broiler chicks were treated with or without dietary LA at 50 mg/kg. At 30 and 31 d old, isoproterenol (2 mg/kg body weight per h) was continuously infused into a wing vein for 2 h, and changes in plasma glucose, triacylglycerol, and non-esterified fatty acid concentrations were analysed. Isoproterenol increased plasma glucose over basal levels maximally at 60 min. Furthermore, the glucose increase in the LA-treated chickens was 35 % greater than that of the controls at this time. Plasma non-esterified fatty acid and triacylglycerol concentrations were decreased by the isoproterenol infusion, regardless of LA administration. Therefore, the present study suggests that dietary LA has repartitioning effects on energy metabolism in chickens (although this depends on age-related metabolic state) and is a possible facilitator in the β-adrenergic response of plasma glucose to a β-agonist.

Lipoic acid: Plasma metabolites: β-Adrenergic response

Consideration of the energy distribution responsible for carcass leanness is a prerequisite for improvement of animal production efficiency. Commercial broiler stocks that have been bred for meat production possess rapid growth performance but have excessive fat deposition associated with adipose cellularity (Cartwright *et al.* 1988). Compared with mammals, fat deposition in chickens appears to be more dependent on energy distribution in the liver, since most fat synthesis in poultry occurs in this organ (Wellenreiter, 1991). Recently, α-lipoic acid (LA), a B vitamin, has been shown to stimulate energy metabolism in several pathological states. LA, a potent biological antioxidant, generally acts as a cofactor in enzyme systems for energy release (Christensen, 1983). It enhances insulin action in glucose transport and metabolism in insulin-resistant rat skeletal muscle (Jacob *et al.* 1996; Streeper *et al.* 1997). In lipid metabolism, plasma cholesterol and lipoprotein levels in rabbits with experimental atherosclerosis have been reported to be decreased by LA treatment (Ivanov, 1974). However, whether the effects of LA on energy metabolism observed in mammalian species also occur in broiler chickens has not yet been investigated.

Furthermore, although this vitamin has been shown to be associated with insulin and thyroid hormones (Segermann *et al.* 1991; Streeper *et al.* 1997), whether there is an interrelationship with β-adrenergic response is unclear. With regard to repartitioning nutrients in chickens, the metabolic response to β-agonist supplementation, which stimulates protein deposition and decreases adipose tissue accretion (Dalrymple *et al.* 1984; Weppelman, 1984; Buyse *et al.* 1991), is dependent on nutritional and hormonal states (Hamano *et al.* 1994, 1995, 1998). In addition, the β-adrenergic response of leanness in chickens is thought to be generally weaker than that of mammals (Yang & McElligott, 1989; Wellenreiter, 1991). More recently, a study from this laboratory indicated that injection of thiamin into broiler chicks facilitated the lipolytic response to a β-agonist, clenbuterol, as monitored by plasma non-esterified fatty acids (NEFA) (Hamano *et al.* 1999). Thus, LA may also stimulate the β-adrenergic response in energy metabolism, acting as a cofactor in hormone action.

Abbreviations: BW, body weight; LA, α-lipoic acid; NEFA, non-esterified fatty acids.

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The aim of the present study was to examine the effects of LA on plasma metabolites, hepatic O_2 consumption, and the metabolic response to β-agonist infusion in broiler chickens.

Materials and methods

Expt 1

Animals and diets. Fifty-one 1-d-old female broiler chicks (Ross Strain, Shosan Shoji Co., Tokyo, Japan) were housed in battery brooders maintained at 35°. All birds had free access to tap water and were given *ad libitum* access to a commercial starter diet (230 g crude protein/kg, 12·5 MJ metabolizable energy/kg) until 3 weeks of age; thereafter, a finisher diet (180 g crude protein/kg, 13·2 MJ metabolizable energy/kg) was given. At 12 d old, the birds were randomly allocated into three groups of seventeen chicks each with similar body weight (BW, 268·5 (SE 2·6) g). The animals in each group were housed together in a wire cage and were fed on the diets containing LA (DL-α-thioctic acid; Kanto Chemical, Tokyo, Japan) at 0 (control), 5, or 50 mg/kg throughout the experimental period for 16 or 30 d (as a 2×3 factorial arrangement). The temperature in a windless room with continuous light was maintained at 25°. BW of chickens were measured at 4 (*n* 17) and 6 (*n* 7) weeks of age. Then, five or seven chickens were randomly selected from each group at 4 or 6 weeks of age. A blood sample was obtained from a wing vein, using a heparinized syringe, and centrifuged (2000 ***g***) to obtain plasma. The plasma samples were stored at −20° until chemical analysis for glucose, triacylglycerol, and NEFA. After blood sampling, the chickens were deeply anaesthetized with pentobarbital sodium (Nembutal® Injection, Abbott Laboratories, North Chicago, IL, USA); then, the left side of breast muscle, abdominal fat, and liver were immediately removed and weighed. The liver (*n* 5) was used to monitor *in vitro* O_2 consumption.

Chemical analysis. Plasma metabolites were analysed colorimetrically using enzymic assay kits for glucose (Kyowa Medex, Tokyo, Japan), triacylglycerol (Sanko Chemical, Tokyo, Japan) and NEFA (Kyowa Medex). To determine O_2 consumption, the liver tissue was washed in ice-cold Krebs-Henseleit buffer containing 20 mM-HEPES and 10 mM-D-glucose (pH 7·4) and sliced to less than 0·5 mm thickness. About 50 mg of the sliced tissue was transferred to a glass chamber containing 4 ml air-saturated Krebs-Henseleit buffer at 37°. The hepatic O_2 consumption was polarographically monitored using an O_2 electrode assembly with a Clark-type probe (model 5300, Yellow Springs Instruments Co., Yellow Springs, OH, USA) for 10–15 min.

Statistical analysis. All data were statistically analysed by a two-way ANOVA (Yoshida, 1995). When a significant interaction between age and LA treatment was detected in each variable, a Scheffé test was used to compare the means within each chicken age (StatView, Abacus Concepts Inc., Berkeley, CA, USA).

Expt 2

Animals and diets. Female broiler chicks were housed in battery brooders and randomly assigned to one of two groups at 3 d old. The chicks were fed on the diets (see Expt 1) containing LA at doses of 0 (control) or 50 mg/kg *ad libitum*, and feeding and environmental conditions were similar to those of Expt 1. At 30 and 31 d old, chickens fasted for at least 6 h (control, *n* 5; LA, *n* 6) were randomly selected (880–1180 g live weight) and operated on under local anaesthesia. Two medical catheters (Nipro, Osaka, Japan; 0·48 mm i.d., 0·68 mm o.d.) were inserted into wing or leg veins. One catheter inserted into the wing vein was connected to a peristaltic pump (Atto Co., Tokyo, Japan), and the other was used for blood sampling. Isoproterenol (Sigma, St Louis, MO, USA), a β-agonist, dissolved in saline (9 g NaCl/l), was continuously infused into the wing vein for 2 h at an infusion rate of 2 mg/kg BW per h (as a solution volume of 0·5 ml/kg per h). Blood (1 ml) was sampled twice at 10 min intervals using a heparinized syringe before initiation of the infusion. During the infusion period, blood samples were taken at 30 min intervals. Plasma samples obtained by centrifugation (2000 ***g***) were stored at −20° until chemical analysis for glucose, triacylglycerol, and NEFA. Plasma metabolites were colorimetrically determined using the enzymic assay kits (see Expt 1).

Statistical analysis. Significant responses of plasma metabolites to isoproterenol infusion in each group were confirmed using a one-way ANOVA (Yoshida, 1995). Then, comparison of the values between control and LA-treated groups at each time was made using the *t* test (StatView, Abacus Concepts Inc.).

Results

Expt 1

The effects of LA supplementation on final BW and tissue weights in broilers are shown in Table 1. No significant interaction ($P > 0{\cdot}05$) between LA and chicken age was observed in final BW or tissue weights of breast muscle or liver, independent of the dose levels. A slight decrease in abdominal fat weight occurred only in the 4-week-old chickens that received LA at 50 mg/kg, but this was not significant ($P > 0{\cdot}05$).

The effects of LA on plasma metabolites in chickens are shown in Table 2. While plasma glucose level increased ($P < 0{\cdot}01$) in the 6-week-old chickens compared with the 4-week-old birds, the supplementation with LA did not affect it. Significant interactions between chicken age and LA administration were detected in plasma triacylglycerol ($P < 0{\cdot}01$) and NEFA ($P < 0{\cdot}05$) concentrations. Consequently, the LA administration affected these plasma metabolites only in 6-week-old chickens. Plasma triacylglycerol in the 6-week-old chickens decreased in a dose-dependent manner with the LA treatment, but a statistically significant effect (a 30 % reduction) was detected only in the group treated with LA at 50 mg/kg diet ($P < 0{\cdot}05$). In contrast, an increase of about 45 % ($P < 0{\cdot}05$) in plasma NEFA concentration was observed in the chickens fed on the high-dose LA compared with controls. These results indicate that the effects of LA on plasma lipids were dependent on chicken age.

The effect of LA administration on the rate of hepatic O_2

Table 1. Effects of lipoic acid (LA) on final body weight (BW), and tissue weights of abdominal fat, breast muscle and liver in broiler chickens
(Mean values with their standard errors)

			Final BW† (g)		Abdominal fat weight (g)		Breast muscle weight (g)		Liver weight (g)	
Age	(mg LA/kg diet)	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
4 weeks	0	5	867	20	13·3	1·0	45·1	2·4	19·8	0·3
	5	5	900	10	11·6	0·5	38·8	1·5	22·6	0·9
	50	5	837	14	7·2	0·5	39·4	0·9	18·2	0·7
6 weeks	0	7	1754	34	27·4	2·1	87·4	4·2	36·8	1·3
	5	7	1816	37	28·2	2·0	90·0	5·5	36·3	1·7
	50	7	1766	50	29·4	2·8	88·9	4·3	38·3	1·2
Statistical significance of effect of: (ANOVA)										
Age			***		***		***		***	
LA			NS		NS		NS		NS	
Age×LA			NS		NS		NS		NS	

*** $P < 0·001$.
† Values at 4 weeks of age are the means for seventeen chickens per treatment.

consumption in broilers is shown in Fig. 1. The hepatic O_2 consumption showed a significant interaction between chicken age and LA in the diet ($P < 0·05$). A significant effect of LA on this variable was detected only in the 4-week-old chickens in which a 42 % increase in O_2 consumption was observed in those that received 50 mg LA/kg diet compared with controls ($P < 0·05$). The effect on hepatic O_2 consumption was negated at 6 weeks of age, independent of dosage.

Expt 2

Changes in plasma glucose (mmol/l) in response to isoproterenol are shown in Fig. 2. The dose effect of isoproterenol infusion on plasma glucose was detected in both control ($P < 0·05$) and LA-fed chickens ($P < 0·001$). The enhanced glucose levels in both groups were maximal at 60 min. The glucose response to the infusion from 30 to 90 min was significantly and considerably greater in the LA-fed chickens than in controls. This sensitivity to isoproterenol in the LA group was enhanced by about 35 % maximally at 60 min compared with control ($P < 0·001$). Thereafter, the increased glucose due to isoproterenol rebounded slightly towards the basal levels.

Fig. 3 shows changes in the plasma concentration of triacylglycerol (μmol/l). Plasma triacylglycerol concentration was also influenced by the isoproterenol infusion in controls ($P < 0·05$) and the LA-treated group ($P < 0·01$). Basal levels of plasma triacylglycerol tended to be higher in the LA-treated group than in controls ($P > 0·05$), but in contrast to plasma glucose, decreased from 30 to 120 min following isoproterenol infusion. This response was maximal at 60 min, and the sensitivity to the β-agonist, measured as the degree of reduced concentrations, showed no significant difference between control (51 %) and LA-fed chickens (56 %).

Table 2. Effects of lipoic acid (LA) on plasma concentrations of glucose, triacylglycerol and non-esterified fatty acids (NEFA) in broiler chickens
(Mean values with their standard errors)

			Glucose (mmol/l)		Triacylglycerol (μmol/l)		NEFA (μmol/l)	
Age	(mg LA/kg diet)	*n*	Mean	SE	Mean	SE	Mean	SE
4 weeks	0	5	10·04	0·70	189	20	588	35
	5	5	9·82	0·35	230	17	591	29
	50	5	10·99	0·52	261	21	528	51
6 weeks	0	7	11·60	0·37	397[a]	35	558[a]	23
	5	7	11·65	0·54	308[ab]	20	780[ab]	91
	50	7	10·93	0·33	279[b]	23	812[b]	47
Statistical significance of effect of: (ANOVA)								
Age			**		**		**	
LA			NS		NS		NS	
Age×LA			NS		**		*	

[a,b] Mean values within a column with different superscript letters in each treatment period were significantly different, $P < 0·05$.
* $P < 0·05$; ** $P < 0·01$.

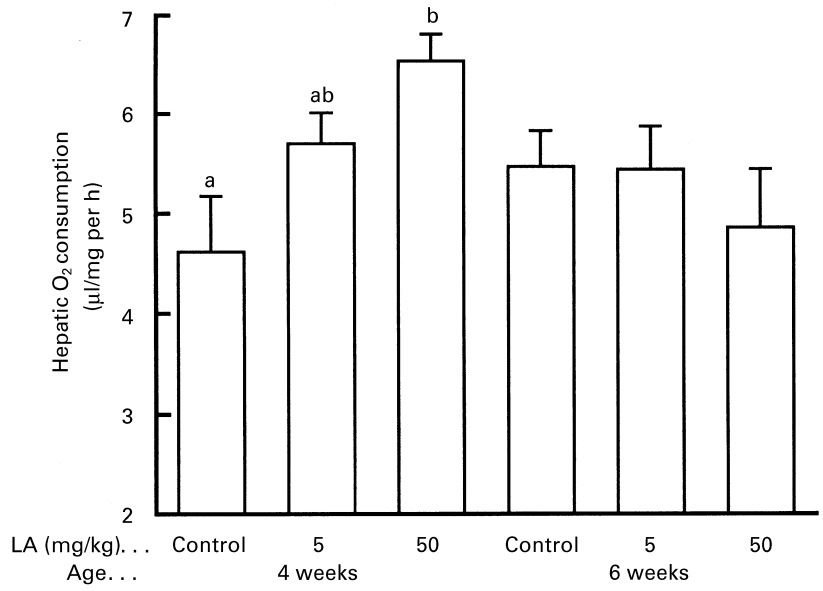

Fig. 1. Effects of lipoic acid (LA) at 5 or 50 mg/kg diet on hepatic oxygen consumption in broilers at 4 and 6 weeks of age. Values are means for five chickens, with their standard errors represented by vertical bars. [a,b] Mean values at 4 weeks not sharing a common letter were significantly different, $P < 0{\cdot}05$. Significance levels of a two-way ANOVA (2 × 3) were as follows: age, $P > 0{\cdot}05$; LA, $P > 0{\cdot}05$; interaction, $P < 0{\cdot}05$.

A dose effect of isoproterenol infusion was observed in plasma NEFA (μmol/l) (control $P < 0{\cdot}01$, LA $P < 0{\cdot}001$; Fig. 4). Isoproterenol infusion resulted in reduced NEFA levels from 30 to 120 min. However, the pronounced depression in this metabolite was not influenced by the LA treatment ($P > 0{\cdot}05$).

Discussion

Many studies have focused on the effect of LA on energy metabolism in pathological states, but there has been little observation of its effect on chicken growth performance and energy metabolism. Gries & Scott (1972) indicated that dietary administration of thiamin increased weight gain with feed consumption in thiamin-deficient chicks. Previously, an increase in breast muscle weight as a proportion of body composition was found when broiler chicks received thiamin injections (Hamano *et al.* 1999). In the present study, LA supplementation had no statistically significant effect on final BW or tissue weights as shown by ANOVA (Table 1), although LA at 50 mg/kg diet slightly reduced adipose tissue accretion of chickens at 4 weeks of age. Consequently, dietary supplementation with LA had no growth promoting effect in chickens.

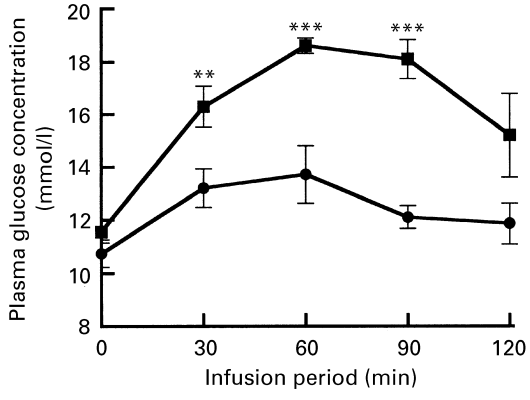

Fig. 2. Effects of isoproterenol infusion on plasma glucose concentrations in chickens with (■) or without (●) lipoic acid (LA) treatment. Values are means for five (control) or six (LA) chickens, with their standard errors represented by vertical bars. Mean values were significantly different from control: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

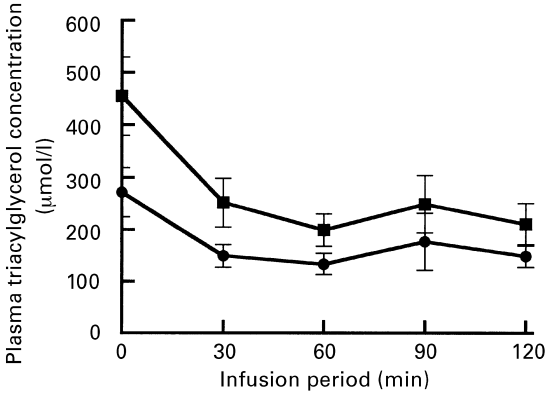

Fig. 3. Effects of isoproterenol infusion on plasma triacylglycerol concentrations in chickens with (■) or without (●) lipoic acid (LA) treatment. Values are means for five (control) or six (LA) chickens, with their standard errors represented by vertical bars.

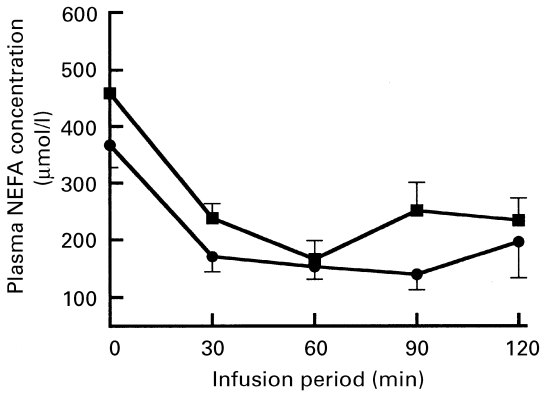

Fig. 4. Effects of isoproterenol infusion on plasma non-esterified fatty acids (NEFA) in chickens with (■) or without (●) lipoic acid (LA) treatment. Values are means for five (control) or six (LA) chickens, with their standard errors represented by vertical bars.

The administration of LA to streptozotocin-diabetic rats has been reported to reduce blood glucose concentration and to increase content of the glucose transporter (GLUT-4) protein in the skeletal muscle (Khamaisi *et al.* 1997). Plasma glucose level increased in the 6-week-old chickens, but the dietary LA did not affect it. In rats, the long-term LA treatment reduced serum triacylglycerol levels (Segermann *et al.* 1991). Ivanov (1974) reported that LA administration to rabbits with experimental atherosclerosis decreased plasma cholesterol levels and body fat accretion. In the present study, a significant effect on plasma lipid levels was confirmed only in the 6-week-old chickens (Table 2). The LA administration at 50 mg/kg diet prevented the age-related increase in plasma triacylglycerol concentration. A depression in plasma NEFA level due to LA was reported in insulin-resistant obese rats that possessed high levels of plasma glucose and NEFA (Streeper *et al.* 1997). In this condition, the LA treatment that facilitates insulin action in glucose uptake or oxidation in skeletal muscle (Streeper *et al.* 1997) would result in reduced fatty acid supply as an energy source. The present results showed that plasma NEFA level in the older chickens was increased by LA feeding, while plasma glucose level was unchanged in the LA-treated chickens. However, these lipid responses to LA were not related to adipose tissue accretion as abdominal fat weight was unchanged by LA treatment.

These effects of LA on plasma lipid were further closely associated with chicken age. Vasilatos-Younken (1986) reported that *in vitro* oxidation of palmitate by adipose tissue and of glucose by liver in broilers was highest at 4 weeks and had decreased by 8 weeks of age. Conversion of these substrates into lipids in the tissues was also reduced with increasing age. Release of free fatty acid from adipose tissue (lipolysis) has been shown to be lower in 28-d-old than 7–8-d-old chicks (Goodridge, 1968) which indicates that lipolytic rate declines with ageing. In contrast, increases in plasma triacylglycerol and glucose concentrations were detected in the 6-week-old chickens compared with the younger chickens in the present study. The ability of the 6-week-old chickens to deposit body fat seemed to be higher than that of the 4-week-old birds. Therefore, the present findings indicate that the dietary LA administered to broilers inhibited transport of neutral fat and brought about a concomitant lipolytic response in adipose tissue, but this was dependent on age-related metabolic states for fat accretion. These metabolic states were probably associated, in part, with hormone secretion or tissue sensitivity to hormones (Scanes *et al.* 1984; Vasilatos-Younken, 1986; Buyse *et al.* 1991), but we did not monitor changes in plasma hormone levels.

Dietary LA supplementation stimulated hepatic O_2 consumption in a dose-dependent manner in 4-week-old chickens. At the high dose level, the pronounced increase in O_2 consumption was associated with slightly reduced adipose tissue accretion. Ivanov (1974) found that, in rabbits with experimental atherosclerosis, LA treatment increased the rate of O_2 uptake by liver, heart, and aorta with reduced lipid accretion. Additionally, the LA-induced reductions in serum total cholesterol and β-lipoprotein concentrations were considerably dependent on an intensification of oxidative processes in these tissues. However, in the present study, the enhancement of hepatic O_2 consumption was an independent response of liver and resulted in no alteration of plasma metabolites.

Although LA supplementation increased energy metabolism concurrently with chicken age, it is difficult to separate the effects of LA and ageing. The experimental design did not distinguish dose effect from length of treatment. Although LA at 5 mg/kg diet was not sufficient to bring about apparent metabolic responses, a significant effect on plasma metabolites was observed at the level of 50 mg/kg. Spence & McCormick (1976) reported that about 80 % of radiolabelled LA (intraperitoneal administration) was recovered in rat urine within 24 h. Other studies have used rats treated with high levels of LA, i.e., daily intraperitoneal injection at 7·5 mg/100 g BW (Segermann *et al.* 1991) or 15–100 mg/kg BW (Streeper *et al.* 1997). In the present study, net LA intake per day varied with feed intake so it was less than 5 or 50 mg/kg BW, although how much less is not clear because feed intake was not measured. Orally administered LA is absorbed almost quantitatively (Peter & Borbe, 1995). The rate of disappearance of LA (half-life) from plasma did not greatly differ between oral and intravenous doses (Peter & Borbe, 1995). In the present study, the dose-dependent LA effect might have been associated with its transport or utilization in tissues rather than absorption in the gastrointestinal tract. Even though the metabolic effect of LA (50 mg/kg) on energy distribution depended on chicken age, a dietary level of more than 50 mg/kg may be necessary to improve growth performance or inhibit adipose tissue accretion. Therefore, further experimental studies are required.

Recent studies have reported interrelationships between LA supplementation and hormonal regulation (thyroid hormones or insulin) of energy metabolism (Segermann *et al.* 1991; Streeper *et al.* 1997). The increased blood glucose level due to isoproterenol, when given intensively, is generally transient and small (Yang & McElligott, 1989; Reeds & Mersmann, 1991). A similar response was also confirmed in control chickens (Fig. 2). On the other hand, β-adrenergic responsiveness of plasma glucose to isoproterenol infusion increased surprisingly in chickens that received LA, although no metabolic response to LA alone

was observed at 4 weeks of age. The pronounced increase in plasma glucose must be attributable to stimulated glycogenolysis. While LA might also stimulate skeletal muscle glucose transport directly (Henriksen *et al.* 1997), glucose mobilization (increased glucose level) should have priority over glucose uptake in tissue response to LA. Thus, in the present study, LA interacted with adrenergic stimulation of glycogenolysis or aerobic glycolysis.

For plasma triacylglycerol, the basal concentrations in Expt 1 were slightly higher than those in Expt 2. While the cause was unclear, this experiment was able, at least, to confirm significant changes in plasma triacylglycerol and NEFA concentrations following isoproterenol infusion and to show no interrelationship in its response to LA administration. The isoproterenol infusion reduced both lipids in contrast to the increased glucose level. β-Agonist administration has been shown to induce the lipolytic response and to reduce lipoprotein level (Buyse *et al.* 1991; Reeds & Mersmann, 1991). More recently, a study from our laboratory found that thiamin injection markedly stimulated plasma NEFA levels in broiler chicks treated with a β-agonist, clenbuterol (Hamano *et al.* 1999). However, the dietary LA did not stimulate lipolysis in the isoproterenol challenge. Moreover, the rapid response to the β-agonist in the chicken was different from that in mammalian species. Concomitant increases in plasma glucose and NEFA following isoproterenol injection were confirmed in cows (Chilliard & Ottou, 1995). Thus, decreases in both plasma NEFA and triacylglycerol concentrations may have been brought about indirectly by the intensively enhanced glucose level rather than by the direct effect of isoproterenol on the tissue response. For example, insulin secretion stimulated by the elevated glucose level also might be responsible, in part, for the depression of plasma lipids following isoproterenol infusion.

In animal production, β-agonists have been determined to be potent growth promoters leading to increased skeletal muscle mass and protein accretion and/or decreased fat deposition. The β-adrenergic responsiveness is considered, in general, to be dependent on β-adrenergic receptor density (Stiles, 1991; Kim *et al.* 1992). Further, chickens have been shown to be insensitive to β-agonist administration (Weppelman, 1984; Wellenreiter, 1991). More recently, nutritional factors such as level of dietary protein and energy, and thiamin administration were shown indirectly to inhibit or improve the repartitioning impact of a β-agonist, clenbuterol, in chickens (Hamano *et al.* 1994, 1998, 1999). Vitamins including LA that act as cofactors in enzyme systems catalysing metabolic processes might be prerequisites for enhancing the subsequent response to β-agonists in the tissues.

Therefore, the present study suggests that dietary LA has repartitioning effects on energy metabolism in chickens, (although this depends on age-related metabolic state), and is a possible facilitator in the β-adrenergic response of plasma glucose to a β-agonist.

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