Change in Triacylglycerol Hydrolase Activity in Hen Liver with Age, and by Estradiol Administration to Male Chicks

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Most of the triacylglycerol hydrolase activity in the hen liver showed its maximum activity at pH 6.0 and at 40°C. The hydrolase activity was markedly inhibited by serum of the laying hen. At 145 and 167 days of age, the activity was markedly decreased to less than one-half that in the young hen. The hydrolase activity in cockerel was monitored to compare with that of hen. In cockerels, the hydrolase activity tended to increase with age of days. On estrogenization of the male chicks, the hydrolase activity in the liver was significantly decreased in contrast to an increase in lipid content in the liver. These observations suggest that the degradation of triacylglycerol in the liver is regulated by estradiol with the increase in lipogenesis for egg production.

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Key words : triacylglycerol hydrolase, liver, hen, cockerel, estradiol

Introduction

The laying hen produces a large amount of lipid in the liver for egg production. Triacylglycerol (TG) is the major component of yolk lipid (BALNAVE, 1968 ; FUJII *et al.*, 1985). Knowledge concerning TG synthesis in the liver of hens have increased over the past 20 years (HAWKINS *et al.*, 1966 ; KUDZMA *et al.*, 1975 ; HASEGAWA *et al.*, 1982). However, little is known about the changes in degradation of TG in the liver of hens associated with the initiation of egg production.

Lysosomal acid TG lipase in mammals is suggested to be important not only to hydrolyze the intracellular lipid, but also to hydrolyze lipids in lipoprotein remnants (NILSSON-EHLE *et al.*, 1980). DEBEER *et al.* (1979) have proposed that only lysosome particles have intracellular lipase in the rat hepatocytes. The acid lipases in the liver of mammals are suggested to serve a central role in cellular lipid metabolism (HAYASE and TAPPEL, 1970 ; TENG and KAPLAN, 1974 ; GOLDSTEIN *et al.*, 1975 ; BROWN and GOLDSTEIN, 1976). However, there have been few studies in the TG hydrolase activity in the liver of birds. We reported previously some properties of acid lipase in the defatted liver of the hen (FUJII *et al.*, 1984 a), and the inhibitor in chicken serum, which inhibited the acid TG lipase in chicken liver (FUJII *et al.*, 1990).

In this paper, we described the change in the TG hydrolase activity in the hen liver with age. The change in the activity of the cockerel liver was also examined. The effect of estrogenization on the TG hydrolase in the liver of male chicks was also examined.

Materials and Methods

Glycerol tri [$1\text{-}^{14}\text{C}$] oleate (50 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, UK. Unlabeled TG and oleate were from Sigma Chemical Co. St. Louis, MO. 2,5-Diphenyl-oxazole (PPO) and 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene (POPOP) were from Nacalai Tesque Inc. Kyoto. All other chemicals were of analytical grade.

Birds

White leghorn pullets and cockerels (30 days of age) were housed in individual cages. They were subjected to 14-h light per day throughout the experiment and fed the experimental diet (Table 1). After the birds were killed about at AM 11 of 97, 118, 145 and 167 days of age (6 birds/sex/age), the livers were obtained and assayed for the TG hydrolase activity and lipid content.

Lipid extraction

A part of liver was lyophilized for lipid analysis. Lipid was extracted by the method of FOLCH *et al.* (1957).

Estrogenization

Estrogenization was done according to the method previously reported (FUJII *et al.*, 1984 b ; 1985). Six male chicks (15 days of age) were estrogenized with 6 daily subcutaneous injections of 17β -estradiol (20 mg/kg/day) in 0.1 ml propylene glycol. The chicks were killed 24 h after the final injection, and the livers were obtained. Six control male chicks (15 days of age) received propylene glycol for 6 days.

Table 1. Composition of the experimental diet

Ingredients (g/kg)	
Maize	794
Alfalfa meal	42
Soybean protein (0.9 potency)	125
Lysine	2.7
DL-Methionine	3.1
Vitamine mix ^a	1
Mineral mix ^b	1
Analysis (per kg on air-dry basis)	
Moisture (g)	128.7
Crude protein ($\text{N} \times 6.25$, g)	180.3
Crude fat (g)	31.7
Crude fiber (g)	16.2
Crude ash (g)	41.7

^a Supplied per kg of diet : choline-HCl 730 mg, folic acid 0.5 mg, biotin 0.03 mg, retinol 6240 I.U., cholecalciferol 1248 I.U., α -tocopherol 9.3 mg, menadiol 1.3 mg, riboflavin 2.5 mg, pyridoxine 0.3 mg, cobalamin 12.5 μ g, and pantothenol 1.5 mg.

^b Supplied per kg of diet : CaCO_3 6.82 g, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ 21.23 g, NaCl 3.63 g, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 0.21 g, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 127 mg, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 190 mg, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 7.49 mg, NaIO₃ 0.48 mg and H_2SeO_3 0.19 mg.

Assay of TG hydrolase activity

The liver was homogenized in 0.01 M Tris-HCl buffer (pH 7.5) with a Waring blender, and assayed for the TG hydrolase activity with glycerol tri [1-¹⁴C] oleate as reported previously (FUJII *et al.*, 1984 b). Briefly, the assay mixture contained, in a total volume of 0.8 ml, 0.5 ml of 0.05 M citrate-0.1 M disodium phosphate buffer (pH 6.0), 0.1 ml of 4.7 mM glycerol tri [1-¹⁴C] oleate (5×10^5 cpm per assay tube), and the liver homogenate. After incubation at 37°C for 60 min, 3 ml of chloroform-methanol-benzene (2 : 2.4 : 1, by vol) and 0.1 ml of 1N NaOH were added. The mixture was vigorously shaken using a Vortex mixer. Radioactivity in an aliquot of the upper layer was measured. One unit of the activity was defined as the amount of protein that liberated 1 nmole of fatty acid per min.

Statistical analysis

Statistical significance of differences was assessed by Duncan's multiple-range test or Student's *t*-test.

Results*Properties of TG hydrolase*

In the hen liver, most of the TG hydrolase activity showed its maximum activity at pH 6.0, and at 40°C. Very little alkaline TG hydrolase activity was detected. The similar profiles of the activities were also found in the livers of the cockerels, chicks, pullets, and estrogenized chicks. Serum of the laying hen inhibited markedly the TG hydrolase activity (Table 2). The sera of cockerel and bovine showed less inhibitory activities in comparison to hen serum.

Change in TG hydrolase activity of hen liver with age

Table 2. Effects of sera on the TG hydrolase activity^a in the liver of the pullets

Additives	Concentration (%)	Relative activity (%)
None	—	100 ^e
Laying hen serum ^b	1.25	26
	2.5	6
	6.25	2
	1.25	82
Cockerel serum ^c	2.5	61
	6.25	27
	2.5	70
Bovine serum ^d	6.25	45

^a The TG hydrolase activity in the livers obtained from two pullets (about 100 days of ages) was 12.2 units/mg protein.

^b The pooled serum obtained from the three laying hens (White Leghorn) of about 280-300 days of ages.

^c The pooled serum obtained from the two cockerels (White Leghorn) of about 300 days of ages.

^d The bovine serum purchased from Handai Biken, Osaka.

^e The mean values of three assays.

The average age in day of the hens for the first egg production was 147 days, while 50% egg production was reached at 149 days of age. At 145 days, two hens had already laid the first egg, but the others did not at that time. At 153 days, the last hen started to lay the first egg. The lipid content in the liver of the hen increased significantly with age (Table 3). In contrast, at 145 and 167 days of age, the TG hydrolase activities in the hen liver were markedly decreased to less than one-half those of the younger hens.

Change in TG hydrolase activity in cockerel liver

Lipid content and TG hydrolase activity in the liver of cockerel were examined at the same ages as those of hens. In the cockerel, the lipid content in the liver was almost constant during the period tested, suggesting that lipid synthesis didn't increase with age in day (Table 4). The TG hydrolase activity in the liver rather increased at 145 and 167 days of age.

Effect of estrogenization on TG hydrolase activity of male chick

The liver weight of the estrogenized birds was about 1.5 times that of control birds (Table 5). The lipid content in the liver was also more than 2 times that of control birds, suggesting the increased lipogenesis on estrogenization. In contrast, the TG hydrolase activity decreased in the liver of estrogenized birds compared to those of the controls.

Table 3. Changes in lipid content and TG hydrolase activity in the hen liver on coming to egg production

Age (days)	No.	Body wt. (kg)	Liver wt. (g)	Liver lipid (mg/g liver)	TG hydrolase activity (units/g liver)
97	6	1.05±0.04 ^a	28.6±3.4 ^a	52.7±8.5 ^a	441±60 ^a
118	6	1.42±0.07 ^b	38.4±4.8 ^b	66.9±12.3 ^{a,b}	379±39 ^a
145	6	1.49±0.05 ^{b,c}	38.8±4.1 ^b	88.3±22.4 ^b	163±62 ^b
167	6	1.52±0.08 ^c	39.3±3.1 ^b	151.8±38.2 ^c	119±40 ^b

Values are means±standard error of mean.

^{a, b, c} Values with the same superscript letter are not significantly different ($p < 0.05$) by Duncan's multiple-range test.

Table 4. Changes in lipid content and TG hydrolase activity in the cockerel liver with age

Age (days)	No.	Body wt. (kg)	Liver wt. (g)	Liver lipid (mg/g liver)	TG hydrolase activity (units/g liver)
97	6	1.42±0.05 ^a	39.9±3.1 ^a	59.2±11.2 ^a	474±91 ^{a, b}
118	6	1.51±0.09 ^b	35.3±3.1 ^b	62.8± 2.5 ^a	414±29 ^a
145	6	1.92±0.06 ^c	30.7±1.5 ^c	50.9± 3.7 ^a	514±37 ^{b, c}
167	6	2.05±0.09 ^d	31.1±2.5 ^c	55.4± 5.8 ^a	559±69 ^c

Values are means±standard error of mean.

^{a, b, c, d}; Values with the same superscript letter are not significantly different ($p < 0.05$) by Duncan's multiple-range test.

Table 5. Effect of a 6-day administration of 17 β -estradiol on male chicks

	Control chicks (n=6)	Estrogenized chicks (n=6)
Body wt, initial, g	111.8 $\pm$ 2.8	117.7 $\pm$ 1.2
final, g	173.0 $\pm$ 5.1	178.7 $\pm$ 5.1
gain, g	61.3 $\pm$ 3.5	61.0 $\pm$ 5.4
Food intake, g	139.2 $\pm$ 5.7	158.1 $\pm$ 7.7
Liver wt, g	7.6 $\pm$ 0.8	11.0 $\pm$ 1.2 ^a
Liver lipid, mg/g liver	52.3 $\pm$ 1.7	117.7 $\pm$ 15.2 ^a
Serum lipid, mg/ml serum	14.1 $\pm$ 0.6	131.7 $\pm$ 3.8 ^a
TG hydrolase activity units/g liver wt	575 $\pm$ 56	207 $\pm$ 43 ^a

Values are means $\pm$ standard error of mean.

^aSignificant at $p < 0.05$ in comparison to control by Student's t-test.

Discussion

We found that the TG hydrolase activity in the hen liver was markedly decreased with age. At that time, the marked increase in lipid content in liver was found, suggesting an acceleration of lipogenesis upon egg production as reported previously (HAWKINS and HEALD, 1966 ; BALNAVE, 1968 ; KUDMA *et al.*, 1975 ; HASEGAWA *et al.*, 1982). In the liver of cockerel, the TG hydrolase activity did not decrease with age, but rather increased. The difference in the changes in the TG hydrolase activity between the hen and cockerel should have some relation to the egg production. By estrogenization of the male chicks, the lipid content in liver was markedly increased. On contrast, the TG hydrolysis activity in the liver was markedly decreased, showing that estrogenization occurred the decrease in the lipolytic function with the increase of lipogenesis in liver. The administration of 17 β -estradiol is well known to cause an increase in lipogenesis (BALNAVE, 1968 ; KUDZMA *et al.*, 1975 ; HASEGAWA *et al.*, 1982). The concentration of estradiol in serum of hen increased before the onset of laying and reached a maximum a few days before the onset of laying (KUDZMA *et al.*, 1975 ; FUJII *et al.*, 1984 b), probably causing the marked increase in lipogenesis for egg production (BALNAVE, 1968 ; HEALD and BADMAN, 1963). As the result, the lipid content increased markedly at that time. In contrast, the TG hydrolase activity was remarkably decreased with coming to the onset of egg production in hen, and on administration of estradiol in male chicks (Table 3, 5). From the above observations, there should be some systems for accumulation of the lipid for egg production in the hens. When the lipogenesis is accelerated by the functions of estradiol, the TG hydrolase activity, as a lipolytic function, in the liver should be controlled to be decreased. Serum of the laying hen markedly blocked the hydrolase activity, suggesting an inhibitory material in the hen serum. There have been several reports concerning the inhibitory activity of serum against the acid TG lipases in fibroblast (GORIN *et al.*, 1982), defatted liver (FUJII *et al.*, 1984 a) and erythrocytes (FUJII *et al.*, 1986). We have highly purified the lipase inhibitor from cockerel plasma (FUJII *et al.*, 1990), and found that the properties of the inhibitor were similar to those of HDL apo A-I (FUJII *et al.*, 1996). Further study is necessary to

determine if the decrease in the TG hydrolase activity in the liver is due to the decrease in the amount of TG hydrolase itself or the increase in amount of inhibitor of the TG hydrolase.

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ニワトリ肝臓のトリアシルグリセロール分解活性の日令による変化と、雄ヒナにエストラジオール投与時の活性の変化

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鶏肝臓に存在するトリアシルグリセロール (TG) 分解活性のほとんどのものは反応至適 pH 6.0, 至適温度 40°C を示し, この分解活性は産卵鶏血清で強く阻害された。この活性は若齢期の雌鶏では高い活性を示すが, 145, 167 日令では若齢期の雌鶏の 2 分の 1 以下へ著しく低下した。比較のため雄鶏でも検討したが, 雄鶏ではむしろ日令と共に増加する傾向があった。雄ヒナにエストラジオールを投与すると, 血清, 肝臓の脂質含量は著し

く増加したが, 肝臓の TG 分解活性は著しく抑制されていた。以上より, エストラジオールにより産卵のための脂質合成の亢進と同時に, 脂質分解の抑制が行われていると考えられた。

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