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The Effect of Choline Deficiency on Lipid Metabolism in Chicks

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Abstract

In the experiment in which young chicks were fed the semisynthetic diet devoid of choline or the same diet with butanolamine supplementation, the weight gain was decreased significantly accompanied by the reduction of feed consumption in choline deficient chicks as compared to control chicks. However, the overall effects of choline deficiency on the relative liver weight, lipid contents of liver, and plasma lipid and lipoprotein levels were not observed, nor was the response to choline deficiency on the incorporation of ^{14}C-oleic acid into lipids in the liver microsomes. When hyperlipidemia was induced by estrogen treatment, the liver lipids, as well as relative liver weight, showed a tendency to be increased only in the chicks fed the semisynthetic diet devoid of choline with butanolamine supplementation. And the magnitude of elevation of VLDL lipids by estrogen treatment was the lowest in the above group. These results indicated that young chicks were not able to synthesize considerable choline for normal growth ; nevertheless, the release of VLDL by hepatocytes was performed normally. But it was also implied that there might be some problems of VLDL release under the condition of hyperlipidemia in chicks in choline deficiency accelerated by butanolamine supplementation.

Key words : young chicks, choline deficiency, ^{14}C-oleic acids, VLDL

INTRODUCTION

The lipotropic action of choline has been demonstrated by Best et al.[1] and confirmed by many investigators in rats[2~4] and dog[5]. Best et al.[6] also established the dose-response basis of choline in controlling the level of liver fat. Thereafter, it has been suggested that choline deficiency produces significant decreases in plasma lipids due to an impairment of hepatic lipids released into plasma[7,8]. The impairment of hepatic lipids released is believed to arise from a reduction in choline-containing phosphatides which are required for the assembly and transport of plasma lipoproteins[9,10].

However, Ketola and Nesheim[11] emphasized the species differences in choline metabolism, especially the biosynthetic ability for choline between chicks and certain mammals. The dietary requirement for choline can not be replaced by high levels of dietary methionine or other methyl donors in chicks[2,12~14]. This is in contrast to the situation in growing mammals such as rats or dogs. It has also been known that the dietary requirement for choline of chicks is age-dependent. For the first few weeks of life young chicks very readily demonstrate choline requirement, but after eight weeks of age, dietary choline requirement is difficult to show[15~17]. Therefore, it is good to use young chicks as an animal model for choline metabolism. Many researchers have studied the relation between choline and egg production in laying hens. However, there have been few investigations using chicks concerning the effects of choline deficiency on lipid metabolism. A few reports[17,18] concerning liver lipids

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have appeared in the literature.

In the present study, we intended to determine the effects of dietary choline deficiency in normal young chicks as well as those in hyperlipidemia condition. For this purpose, one week-old chicks were fed the semisynthetic diet devoid of choline in order to induce choline deficiency, or fed the same diet with a choline analog, l-2-amino-1-butanol supplementation in order to acceralate the choline deficiency. And half of the chicks were treated with estrogen in order to induce hyperlipidemia. We determined the changes in plasma lipids, lipoproteins, liver lipids, and the ability of lipogenesis expressed by the incorporation of radioactivity from ^{14}C-oleic acid in the liver microsomes.

MATERIALS AND METHODS

Animal and diet

Thirty six one week-old male chicks from the poultry farm at University of Illinois, were randomly divided into three groups and raised in wire cages under a constant lighting cycle (7 : 00~21 : 00 hr.). Chicks in the first group (Choline-free ; CF) were fed the semisynthetic diet (Table 1) devoid of choline for a period of two weeks. Those in the second (Choline ; C) and the third group (Choline-free+butanolamine ; CFB) were fed the same diet and provided supplemental choline and l-2-amino-1-butanol, respectively, during the experimental periods. Choline and butanolamine were supplemented through drinking water containing 695mg and 500mg per liter in water, respectively. Water was provided by a specially devised bottle. Feed consumption was recorded every three days, and water consumption was recorded daily. Half of each group of chicks was treated with estrogen two days before ending the experiment. Estrogen (17-β-estradiol, 20mg/kg Bd. wt.) dissolved in propylene glycol was injected subcutaneously into each bird. The other half of each group of chicks was injected the carrier alone. At the end of the experiment, blood and liver samples were collected after sixteen hours fasting.

Table 1. Composition of diet

Constituents	Percentage of diet
Casein[1]	22.0
Corn oil[2]	1.0
Dextrose	65.73
Solka floc[3]	3.0
Arginine[4]	0.9
Glycine[5]	1.5
Mineral mixture[6]	5.37
Vitamin mixture[7]	0.2

[1]Nutritional Biochemical Co., Cleveland, OH
[2]Mazola corn oil, CPC international Inc., Engldwood Cliffs, NJ
[3]Brown Co., Berlin, NH
[4,5]Sigma Chemical Co., St. Louis, MO
[6,7]The mixtures of mineral and vitamin are based on the patterns of Baker et al.[19]

Separation of lipoproteins

Blood samples were drawn from heart into tubes containing ethylenediamine tetraacetic acid (EDTA, 1.0mg/ml blood). Plasma was obtained by centrifugation at 1,000 x g for 30min at 4° C. Separation of lipoproteins was carried out by sequential preparative ultracentrifugation with Ti 40.3 rotor at 10° C in a Beckman Model L5-50 Ultracentrifuge as described by Havel et al.[20] Three lipoprotein fractions were isolated: very low density lipoprotein (VLDL ; $d<1.006$g/ml), low density lipoprotein (LDL ; $1.006<d<1.063$g/ml) and high density lipoprotein (HDL ; $1.063<d<1.21$g/ml).

Determination of lipoprotein lipids

The levels of triglyceride (TG), total cholesterol (CH) and phospholipids (PL) were analyzed. Triglyceride was measured by the method of Foster and Dunn[21]. Total cholesterol was determined enzymatically[22] by using cholesterol C11 reagent (Wako Pure Chemical Industries, Ltd. Osaka). Determination of PL was carried out by the method of Eng and Noble[23].

Preparation of liver microsomes

After a blood sample was drawn, the liver was re-

moved immediately, rinsed in ice-cold 0.9% saline solution, blotted on filter paper and weighed. To prepare liver microsomes, each small portion of freshly excised liver from three birds, respectively, was pooled and homogenized with 5ml of homogenizing solution (pH 7.4) containing 0.25M sucrose, 0.001M EDTA and 0.001M dithiothreitol using a tissue homogenizer equipped with a Polytron (Brinkman Instruments, Westburry, NY). The crude homogenate was centrifuged at 10,000 x g for 30 min at 4° C. The resulting supernatant was recentrifuged under the same conditions. And the resulting supernatant was then further centrifuged at 105,000 x g for 60min at 4° C. The resulting 105,000 x g pellet was suspended in 5ml of the same buffer. Protein content of the microsomal preparation was determined by the method of Lowry et al.[24]

Incubation system of liver microsomes

The incubation system contained in 1.5ml : prepared microsomes 1.2mg of protein ; 100μmoles of potassium phosphate buffer (pH 7.4) ; 0.2mg of bovine serum albumin ; 0.1nmoles of CoASH ; 2μ moles of ATP ; 2μmoles of $MgCl_2$; 50nmoles of ^{14}C-oleate (0.4μCi) in acetone. Incubation was carried out for 1hr at 37° C with shaking. The reaction was stopped by adding 15 ml of chloroform-methanol (2:1, v/v) and the radioactive lipids were extracted three times with Folch solution[25]. Combined solvent was evaporated under a stream of nitrogen and the residue was redissolved in a small amount of chloroform. An aliquot of lipid extract was used to determine the incorporation of radioactivity from ^{14}C-oleate into microsomal lipids.

Determination of radioactivity incorporation

In order to determine radioactivity incorporation, the lipids extract was seperated by thin-layer chromatography on instant Polysilicic Acid Gel (ITLC : Gelman Instrument Co., Michigan) with petroleum ether-diethyl ether-acetic acid (90:10:1, v/v/v). The plate was exposed to iodine vapor to make the spots of neutral lipids visible. The portions of plate containing TG and cholesteryl ester (CE) were scraped individually, transfered into scintillation vials, and 5ml of toluene cocktail (0.5% PPO and 0.03% dimethyl POPOP) was added to each vial. The radioactivity was counted by a Beckman LS 2800 Liquid Scintillation Spectrometer. For quantitation of radioactivity incorporation into PL, the lipids extract was separated by two-dimensional TLC on Silica Gel H (300μm thick) according to Glaser et al.[26] Solvent system consisting of chloroform-methanol-water (65:25:4, v/v/v) was used in the first dimension and n-butanol-glacial acetic acid-water (6:2:2, v/v/v) was used in the second dimension. The spots of PL were visualized by exposure to iodine vapor. The silica corresponding to each spot was scraped from the plate into a scintillation vial and 5ml of toluene cocktail was added. The radioactivity was measured after two days, when the cpm reached a constant value.

Determination of liver lipids

The liver levels of TG, CH and PL were analyzed using the methods described for lipoprotein lipids above, after total lipids were extracted from the suitable portion of liver by the method of Folch et al.[25]

Statistics

Data were analysed by one-way analysis of variance and Fisher PLSD multiple comparison test.

RESULTS

The results of growth performances, liver weight and lipids, and plasma lipids in the three dietary treatment groups with or without estrogen treatment are presented in Table 2. Body weight gain was not significantly different among the three groups without estrogen treatment ($p<0.05$) ; The body weight gain in CFB group was the lowest, followed by CF group. The consumptions of feed as well as water in CFB group were less than those both in C and CF groups, but chicks in CF group consumed the same amount of feed and water as C group. So the feed conversion ratio in CFB group was the greatest, fol-

lowed by CF group. Estrogen treatment did not affect growth performances.

Relative liver weight was not show significantly different among the three groups without estrogen treatment ($p<0.05$). Estrogen treatment resulted in marked elevation of the relative liver weight only in CFB group ($p<0.05$). That in both C and CF groups showed a tendency to be increased by estrogen treatment, but not significant. Liver lipid levels did not significantly differ among the three groups without estrogen treatment ($p<0.05$). Estrogen treatment resulted in significant elevation of liver lipids except TG only in CFB group ($p<0.05$). Those in both C and CF groups showed a tendency to be increased by estrogen treatment, but not significant.

No significant differences were shown in plasma lipid levels among the three groups without estrogen treatment ($p<0.05$). But not significant, those levels in both CF and CFB groups showed a tendency to be decreased, especially in CFB group. Estrogen treatment induced hyperlipidemia as expected by elevation of TG as well as CH and PL. The magnitude of elevation was somewhat greater in both CF and CFB groups than that in C group, but not significant.

The responses of plasma lipoprotein lipid levels in the three groups with or without estrogen treatment are presented in Table 3. Lipoprotein lipid levels did not show significant differences, except in both TG and PL of HDL among the three groups without estrogen treatment ($p<0.05$) ; The levels of TG and PL of HDL in CFB group were lower than those in both C and CF groups. All of the lipoprotein lipid levels in CF as well as in CFB group, in general, were lower than those in C group. Estrogen treatment resulted in marked elevation of TG, CH and PL levels of both VLDL and LDL in all of the three groups ($p<0.05$). But the extent of elevation was different; That of VLDL lipids was significantly greater in CF group, and that of LDL lipids was significantly great in CFB group ($p<0.05$). However, the levels of HDL lipid, especially CH, were reduced significantly, except in one case by estrogen treatment ($p<0.05$). The extent of reduction in CFB group was significantly greater than that in both C and CF groups.

The data of radioactivity incorporation from ^{14}C-oleic acid into liver microsomal lipids in the three groups with or without estrogen treatment are given in Table 4. The radioactivity was incorporated into TG and cholesteryl ester (CE) as well as phosphatidyl choline (PC), phosphatidyl ethanolamine (PE)

Table 2. Effects of treatment on growth performances, liver weight and lipids, and plasma lipids in chicks[1-3]

Dietary treatment	C		CF		CFB	
Estrogen treatment	−	+	−	+	−	+
Wt. gain (g/day)	12.3±0.8^{a}	10.2±0.8^{ab}	8.5±0.7^{b}	8.8±0.7^{b}	6.2±0.8^{c}	6.0±0.8^{c}
Feed intake (g/day)	21.2	21.3	21.5	23.6	18.0	17.7
Water intake (ml/day)	56.1	58.2	59.5	57.9	30.9	32.6
Feed conversion ratio	1.72	2.09	2.53	2.68	2.90	2.95
Liver wt. (g/kg Bd. wt.)	3.1±0.1^{c}	3.5±0.1^{abc}	3.3±0.1^{abc}	3.4±0.1^{b}	3.2±0.1^{c}	3.9±0.1^{a}
Liver lipids (mg/g wet tissue)						
TG	6.0±0.7^{ab}	6.9±1.2^{ab}	5.5±0.4^{b}	6.5±1.1^{ab}	8.6±1.7^{ab}	11.4±4.0^{a}
CH	3.5±0.1^{ab}	3.4±0.1^{ab}	3.3±0.1^{b}	3.9±0.6^{ab}	3.0±0.1^{b}	3.7±0.2^{a}
PL	26.2±0.6^{ab}	26.1±0.4^{a}	24.8±0.4^{ab}	26.5±0.3^{a}	24.3±0.4^{b}	25.9±1.0^{a}
Plasma lipids (mg/100 ml)						
TG	59.5±1.7^{c}	808.1±78.1^{b}	50.7±2.8^{c}	1008.1±23.1^{a}	40.7±4.3^{c}	836.3±6.3^{b}
CH	180.7±6.1^{d}	256.7±1.5^{c}	177.7±12.8^{d}	292.6±19.7^{bc}	143.4±23.5^{d}	313.8±14.0^{ab}
PL	305.9±2.0^{c}	555.0±52.8^{b}	271.5±23.3^{c}	704.4±34.6^{a}	229.8±9.8^{c}	470.1±25.4^{b}

[1]Abbreviations : TG, triglyceride ; CH, total cholesterol ; PL, phospholipids

[2]Feed conversion ratio is g feed consumed/ g weight gain

[3]Values are mean±SE. Means (N=6) in horizontal rows not sharing a common superscript letter differ significantly ($p<0.05$)

and phosphatidyl butanolamine (PBA). Radioactivity incorporations into both TG and CE did not significantly differ among the three groups without estrogen treatment ($p<0.05$). However, radioactivity incorporations into subclasses of PL showed some significant differences ($p<0.05$) ; In the case of PC incorporation, that in CF group was significantly greater than that in CFB group. And in the case of PE incorpoation, that in CF group was greater than that in both C and CFB groups, but not significant. Considerable amount of radioactivity was incorporated into PBA in CFB group. Estrogen treatment did not affect radioactivity incorporations into both TG and CE in all of the three groups, but, those in CFB group were slightly greater than those in both C and CF groups. However, estrogen treatment resulted in decreases of radioactivity incorporations into PC, PE and PBA in all groups except into PC in C group. No significant differences in PC/PE ratio were observed among the three groups without estrogen treatment. The PC/PE ratios in both C and CF groups, but not CFB group were raised significantly by estrogen treatment ($p<0.05$).

Table 3. Effects of treatments on lipoprotein lipids in chicks[1,2]

Dietary treatment	C		CF		CFB	
Estrogen treatment	−	+	−	+	−	+
Lipoprotein lipids (mg/100ml plasma)						
VLDL TG	8.8±0.8^{c}	556.7±87.4^{ab}	4.2±0.1^{c}	687.4±76.2^{a}	4.5±1.9^{c}	433.1±15.1^{b}
CH	1.4±0.0^{c}	110.0±20.0^{b}	0.8±0.2^{c}	141.0±11.0^{a}	1.0±0.7^{c}	97.0± 8.0^{b}
PL	2.3±0.3^{c}	242.3±45.9^{ab}	1.4±0.2^{c}	334.1±63.8^{a}	1.3±0.7^{c}	176.0± 7.7^{b}
LDL TG	21.7±3.6^{c}	235.7±18.2^{b}	17.5±1.6^{c}	289.3±52.9^{b}	14.3±1.2^{c}	387.8±21.9^{a}
CH	49.2±1.6^{c}	129.6±17.4^{b}	50.4±8.0^{c}	111.0±29.8^{b}	48.0±4.4^{c}	196.4±18.0^{a}
PL	39.1±0.1^{b}	168.1± 8.5^{a}	37.9±5.5^{b}	194.6±37.1^{a}	33.7±1.8^{b}	228.9±16.7^{a}
HDL TG	29.0±1.1^{a}	25.0±0.8^{b}	29.0± 1.1^{a}	31.3±0.3^{a}	21.8± 2.9^{b}	15.4± 0.5^{c}
CH	130.1±4.5^{a}	35.6±3.7^{bc}	126.6± 5.0^{a}	40.6±0.9^{b}	119.5± 6.0^{a}	20.4± 4.0^{c}
PL	264.5±1.8^{a}	137.7±5.4^{c}	232.1±17.9^{a}	175.7±7.9^{b}	194.4±12.3^{b}	63.8±17.9^{d}

[1]Abbreviations : VLDL, very low density lipoprptein ; LDL, low density lipooprotein ; HDL, high density lipoprotein ; TG, triglyceride ; CH, total cholesterol ; PL, phospholipids

[2]Values are mean±SE for 6 birds by analyses pooled samples from 3 birds, respectively. Means in horizontal rows not sharing a common superscript letter differ significantly ($p<0.05$)

Table 4. Effects of treatments on the incorporation of 1^{4}C-oleic acid into various fractions of microsomal lipids in chicks[1,2]

Dietary treatment	C		CF		CFB	
Estrogen treatment	−	+	−	+	−	+
TG	3.45±0.35^{a}	3.71±0.53^{a}	3.84±0.84^{a}	3.66±0.57^{a}	3.21±0.31^{a}	4.62±0.37^{a}
CE	1.55±0.26^{a}	2.04±0.36^{a}	1.66±0.06^{a}	1.69±0.61^{a}	1.95±0.23^{a}	1.95±0.23^{a}
PL						
PC	5.19±0.47^{abc}	5.94±0.62^{a}	5.61±0.36^{a}	5.49±0.52^{ab}	4.52±0.22^{bc}	4.15±0.46^{c}
PE	3.51±0.42^{ab}	2.89±0.20^{b}	4.34±0.12^{a}	3.17±0.33^{b}	3.53±0.26^{ab}	3.38±0.37^{ab}
PBA					6.39±0.77^{a}	5.58±0.98^{a}
PC/PE ratio	1.50±0.14^{c}	2.05±0.09^{a}	1.30±0.09^{c}	1.75±0.17^{b}	1.30±0.13^{c}	1.23±0.03^{c}

[1]Abbreviations : TG, triglyceride ; CE, cholesteryl ester ; PC, phosphatidyl choline ; PE, phosphatidyl ethanolamine ; PBA, phosphatidyl butanolamine

[2]Values are mean±SE (picomoles of oleic acids incorporated/hr/mg protein) for 6 birds by analyses pooled samples from 3 birds, respectively. Means in horizontal rows not sharing a common superscript letter differ significantly ($p<0.05$)

DISCUSSION

Previous studies using rats and dogs revealed choline deficiency induced fat infiltration in the liver accompanied by hypolipidemia[1,2,5~8]. However, the results of this experiment were in contrast to the above reports. The relative liver weight, liver lipid and plasma lipid levels of the chicks in CF group were similar to those of C group chicks. Even the chicks in CFB group showed no changes. The data of lipoprotein lipid levels also appears to coincide with the data of plasma lipids. That is, choline deficiency did not result in any significant changes in all of the major lipid classes of VLDL, LDL and HDL. But only the chicks in CFB group showed a reduction of both TG and PL of HDL.

Furthermore, when extreme hyperlipidemia was induced by estrogen treatment, the relative liver weights and liver lipid levels of the chicks in CF group were not increased either in comparison with those of C group chicks. But the relative liver weight and liver TG showed a tendency to be increased in the CFB group chicks.

The results of this experiment raised 2 questions. The first question is: are young chicks able to synthesize considerable choline for TG release by hepatocytes? Nesheim et al.[17] reported that very young chicks have a very readily demonstrated choline requirement, but after 8 weeks of age, a dietary choline requirement is difficult to show even for hens laying eggs. Presumably, this is due to the different biosynthetic ability for choline possessed by age. Earlier reports revealed that choline deficiency resulted in low appetite[27], slow growth[12] and perosis[28,29] in growing chicks. The body weight gain in this experiment was apparently reduced by choline deficiency. And the PL content of liver, plasma, and all the lipoprotein fractions showed a tendency to be reduced by choline deficiency. These apparently indicated that chicks in this experiment were not able to synthesize considerable choline for normal growth. But, fatty liver was not induced in choline deficiency. So the second question is raised: is the role of choline on TG release by hepatocytes in chicks different from rats or dogs? Phosphatidylcholine provides an integral part of the structure and assembly system of lipoproteins and the microsomal membranes involved in the assembly and secretion processes of lipoproteins[30]. In rats, PC is synthesized by stepwise methylation of PE when in choline deficiency. Wells and Remy[31] reported that the rate of PC synthesis by using L-methionine was greater in the choline-deficient rat than that in the normal rat.

However, Ketola and Nesheim[11] confirmed the reports by Jukes et al.[13] and Henderson and Henderson[14] that chicks were not able to transfer methyl groups of methionine to choline. They emphasized the species differences in choline metabolism between chicks and mammals. Kushwaha and Jensen[32] concluded that choline was synthesized in Japanese quail through the same pathway as that shown for rats, but quail were not able to meet their choline requirements through de novo synthesis because the rate of the first methylation of PE was slower than that in rats. They[33] also insisted that there were significant differences in activities of methyltransferase activity among species.

In this experiment, the incorporation of oleic acid to lipid classes in the liver microsomes was not affected by dietary choline. This result indicated that PC biosynthesis was not enhanced when in choline deficiency in chicks. Furthermore, the liver fat accumulation did not occur even under the condition of hyperlipidemia. This proved that TG was released normally by hepatocytes in hyperlipidemia as well as under normal conditions. The above results indicated that the role of choline on TG release by hepatocytes in chicks was different from rats. To reveal the above assumption it is necessary to study the content of both PC and the other subclasses of phospholipids of VLDL and to establish the mechanism of assembly and secretion of VLDL in chicks.

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콜린 결핍이 병아리의 지질대사에 미치는 영향

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요 약

1주령 병아리에 무콜린 식이를 급여하거나 무콜린 식이에 butanolamine을 첨가 급여하여 콜린 결핍을 심화시켜, 콜린 결핍이 지질대사에 미치는 영향을 알아보았다. 그 결과 콜린 결핍은 간장 중량, 혈장 지질 및 지단백 수준에 유의적인 영향을 나타내지 않았다. 또한 간세포의 소포체에서 ^{14}C-oleic acid의 지질 혼입율에도 유의적인 영향을 끼치지 않았다. 한편 estrogen을 투여하여 고지혈증을 야기시킨 경우 콜린 결핍군의 VLDL상승 정도가 낮은 경향을 나타내었고 비간장 무게와 간장의 지질함량은 증가하는 경향을 나타내었다. 한편 콜린 결핍은 병아리의 성장을 저해하였다. 성장에 필요한 콜린을 충분히 합성하지 못하는 어린 병아리에 콜린 결핍을 야기시켜 얻어진 이러한 결과는 간세포에서의 VLDL방출에 콜린이 크게 작용하지 않음을 시사하여 주었다. 그러나 고지혈증이 유발된 상황에서 극심한 콜린 결핍은 VLDL방출에 약간의 영향을 끼칠 수도 있음을 나타내었다.