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EFFECT OF OROTIC ACID AND CHOLESTEROL ON THE SYNTHESIS AND COMPOSITION OF CHICKEN (*GALLUS DOMESTICUS*) SERUM LIPOPROTEINS

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Abstract—1. Chickens fed a 1% orotic acid supplement to a control diet, had similar liver and lipoprotein protein and lipid levels as those on the control diet alone. Furthermore, *in vivo* radioactive labelling experiments suggested that orotic acid alone did not influence the synthesis of the serum lipoproteins. In both the control and the orotic acid fed animals no label was incorporated into the serum very low density lipoproteins (VLDL). This lipoprotein fraction was almost non-existent in these 2 dietary groups.

2. Addition of 1% cholesterol (group C) to the control diet of the birds induced a large increase in the VLDL concentration as well as the total cholesterol (TC) concentration of both serum and the liver. Concomitantly, a 60–70% decrease in the phospholipid (PL) along with a smaller decrease in the protein concentration of the serum high density lipoproteins (HDL) was observed.

3. When 1% orotic acid was added to the cholesterol diet only about 35% of the increase over controls seen in the TC level of the VLDL and liver of group C birds was observed. Similarly, the amount of incorporation remaining in the VLDL, 4 hr after injection of label, was also about 35% of the amount that was observed in the group C chickens.

4. The decline in the HDL protein and PL levels observed in the group C animals was not prevented by the addition of orotic acid to the cholesterol diet.

INTRODUCTION

Orotic acid, an intermediate in pyrimidine biosynthesis, produces a severe fatty liver in the rat (Windmueller, 1963). Addition of adenine sulfate or removal of orotic acid from the diet caused the liver lipids to decline to normal values within 7–10 days (Windmueller & Levy, 1967). Most of the liver lipid elevation found in rats fed a 1% orotic acid diet was due to an increased triglyceride level although their total cholesterol levels were 2–4 times greater than those of rats on a control diet (Windmueller, 1964). Liver phospholipid levels were unaffected by addition of orotic acid to the diet.

Within 7 days from initiation of a 1% orotic acid diet, the β-lipoprotein level decreased to less than 1% of normal in rats (Windmueller *et al.*, 1970). Livers from orotic acid-fed rats, perfused with a serum-free medium, released no detectable amounts of β-lipoprotein into the medium (Windmueller & Levy, 1967). Intestinal synthesis of β-lipoprotein, was unaffected by the inclusion of orotic acid in the diet of rats (Windmueller *et al.*, 1970). All the serum lipids decreased several fold within one week of administration of the orotic acid diet (Windmueller, 1964).

In the present studies with cholesterol-fed chickens, in which a cholesterol fatty liver rapidly develops along with a concomitant hypercholesterolemia, the possibility of lowering the serum cholesterol level with an orotic acid dietary supplement was considered. It was reasoned that if the mechanism of lowering serum lipid levels was the same in chickens as in rats, then the addition of orotic acid to the cholesterol diet would decrease the elevated serum cholesterol levels. To this end, both serum and liver total cholesterol levels and incorporation of labelled amino acid into the cholesterol-rich very low density lipoproteins were studied when orotic acid was included in the control or 1% cholesterol diet. The composition and amount of label incorporated in the serum low density and high density lipoprotein fractions were also studied as a function of diet.

EXPERIMENTAL

One-day old cockerels, New Hampshire–Columbian cross, were fed the diets plus water *ad libitum* for 4 or 8 weeks. Four different diets were used, a control, a 1% orotic acid (O), a 1% cholesterol (C) and a 1% cholesterol–1% orotic acid combination (CO). The control diet consisted of 90% basal ration and 10% corn oil (Wesson Oil).

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The group O diet was identical with the control diet except for an additional 1% of orotic acid (General Biochemicals, Chagrin Falls, OH, U.S.A.). The group C diet was the same as the control diet except for an addition of 1% cholesterol (Nutritional Biochemicals Corp., Cleveland, OH, U.S.A.). Lastly, the group CO diet consisted of 1% cholesterol and 1% orotic acid added to the control diet.

The fasted chickens (12–18 hr) were anesthetized using sodium pentobarbital (Diamond Laboratories, Des Moines, IA, U.S.A.) and the blood was obtained by cardiac puncture. Each animal was weighed immediately before sacrifice. The livers were quickly removed, weighed and frozen until needed for chemical analysis. Blood was allowed to clot at room temperature before centrifuging to obtain the serum. The supernatant serum was pipetted off and a merthiolate solution was added as a preservative. The serum lipoproteins were obtained by ultracentrifugal methods previously described (Kruski & Narayan, 1972*a*).

The lipid extraction procedure was accomplished using the method of Folch *et al.* (1957). Protein determinations were accomplished using the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. A modification of this method was used when protein values for very turbid lipoprotein fractions were necessary (Kruski & Narayan, 1972*b*). Phospholipids (PL) were measured using the method of Bartlett (1959) with a standard of dipalmitoyl lecithin (Sigma Chemical Co.). Total cholesterol (TC) was determined according to a modification of the method of Zuckermann & Natelson (1948) using a cholesteryl palmitate (Sigma Chemical Co.) standard. Total lipids (TL) were determined by the method of Amenta (1964) using tripalmitin as the standard.

L-[^{14}C] leucine (uniformly labelled)(International Chemical and Nuclear Corp.) dissolved in Krebs–Ringer bicarbonate buffer was given by intraperitoneal injections to the chickens. A total of 15 μCi was injected into each bird in one injection. By extracting the lipid from the labelled lipoprotein fractions, it was found that the radioactive leucine preferentially (<95%) incorporated into the protein moiety of these lipoproteins. The amount of radioactivity in each lipoprotein fraction was determined as previously described (Kruski & Narayan, 1972*c*).

RESULTS

A comparison of the chickens fed the 4 diets during the 4- or 8-week period indicated no appreciable difference in body weight on any of these rations (Table 1). The liver weight percentage of the total body weight showed no significant differences regardless of the diet consumed by the chickens, although livers from group C animals had consistently larger mean values.

The chickens fed the different diets for 4 weeks, generally had greater liver protein, PL, TL and TC concentrations that the chickens fed the diets for 8 weeks (Table 1). The most significant change in the liver composition among the birds on the different diets was noted in the TC concentration. The liver TC concentration increased 10 and 13 times (both $P < 0.001$) for the chickens fed the group C diet for 4 and 8 weeks, respectively as compared to the control animals. Birds on the group CO diet had elevated TC levels compared to the control animals but the increase was about 65% ($P = 0.01$) less than that of chickens fed the group C diet. Although the TL determination used here is nonspecific for lipids, the sum of the liver PL and TC concentrations was generally close to the TL level indicating that triglyceride levels were probably low in all diet groups. The changes in the liver PL and protein levels were relatively unaffected by the dietary intake of either cholesterol and/or orotic acid.

The serum VLDL was almost non-existent in both control and group O fed animals whereas a very substantial VLDL level was present in animals on the group C and group CO diets for both 4- and 8-week feeding periods (Tables 2 and 3). The addition of orotic acid (group CO) to the group C diet decreased the VLDL level for both 4- and 8-week feeding periods. The VLDL protein, PL, TL and TC levels were 69, 47, 67 and 62% (all $P = 0.01$ except PL were $P = 0.1$), respectively less in birds fed the group CO diet compared to the group C diet for the 4-week period (Table 2). Similar decreases of 68, 64, 64 and 72% (all $P = 0.5$ except TL were $P = 0.1$ and PL which is not significant, $P < 0.1$) were found in the VLDL protein, PL, TL and TC, respectively, levels after 8 weeks duration on the diets (Table 3).

The compositional changes observed in the serum LDL fraction reflects the differences seen in the VLDL fractions of birds on the respective diets. The LDL protein, PL, TL and TC levels are highest in animals fed the group C diet, lowest in the control

Table 1. Liver composition of chickens fed control, 1% cholesterol, 1% orotic acid and 1% cholesterol plus 1% orotic acid diets[a]

Description of diet	Diet (weeks)	Body wt (g)	Liver wt × 100 / Body wt	Protein	TL (mg in g wet wt liver)	PL (mg in g wet wt liver)	TC (mg in g wet wt liver)
Control	4	409 ± 42	2.51 ± 0.13	171.5 ± 22.1	20.8 ± 9.8	18.9 ± 3.0	5.4 ± 1.9
	8	945 ± 86	2.14 ± 0.19	161.1 ± 14.3	19.3 ± 2.4	17.4 ± 2.1	3.8 ± 1.7
1% Orotic acid	4	385 ± 63	2.51 ± 0.32	162.5 ± 17.9	27.2 ± 2.9	13.3 ± 1.3	7.9 ± 4.2
1% Cholesterol	4	463 ± 73	2.95 ± 0.45	150.4 ± 25.7	71.3 ± 20.2	19.5 ± 9.8	56.6 ± 16.6
	8	1049 ± 134	2.78 ± 0.28	132.3 ± 17.4	61.8 ± 6.2	14.3 ± 3.3	47.7 ± 13.5
1% Cholesterol + 1% Orotic acid	4	392 ± 27	2.78 ± 0.16	160.9 ± 24.7	44.8 ± 12.9	18.0 ± 3.1	21.5 ± 10.2
	8	953 ± 131	2.43 ± 0.37	132.4 ± 23.5	32.2 ± 5.9	13.2 ± 2.2	21.7 ± 7.4

[a]These values are the mean ± S.D. for 8 chickens, on each of the 4 week diets and 4 chickens on each of the 8 weeks diets.

Table 2. Composition of lipoproteins from chickens fed control, cholesterol, orotic acid and orotic acid plus cholesterol diets for 4 weeks[a]

Lipoprotein fraction	Diet	Protein	TL	PL	TC
			(mg in 100 ml serum)		
VLDL	Control	0.6 ± 0.2	16.4 ± 11.1	0.7 ± 0.3	1.6 ± 0.5
	C	49.1 ± 24.0	237.7 ± 80.4	47.3 ± 17.2	156.5 ± 58.7
	O	0.5 ± 0.1	6.0 ± 4.6	0.6 ± 0.3	1.3 ± 0.4
	CO	15.3 ± 3.5	79.6 ± 30.4	25.1 ± 10.4	60.0 ± 11.6
LDL	Control	20.5 ± 0.6	20.6 ± 6.5	8.5 ± 2.5	11.8 ± 4.1
	C	44.9 ± 7.6	102.3 ± 32.1	27.7 ± 4.6	78.1 ± 20.3
	O	23.0 ± 1.2	27.2 ± 7.6	10.8 ± 3.0	13.6 ± 2.9
	CO	34.1 ± 4.8	85.0 ± 10.6	26.0 ± 4.2	61.6 ± 12.3
HDL	Control	171.0 ± 27.4	96.8 ± 32.3	73.1 ± 30.0	57.0 ± 20.5
	C	103.5 ± 24.1	62.6 ± 19.3	21.6 ± 10.6	40.2 ± 14.2
	O	164.1 ± 44.7	108.4 ± 38.5	71.4 ± 27.5	57.9 ± 21.7
	CO	91.6 ± 32.4	61.5 ± 14.6	27.8 ± 5.7	38.6 ± 9.5

[a]Each value is the mean ± S.D. of the respective fraction from 4 chickens.

Table 3. Composition of lipoproteins from chickens fed control, cholesterol and orotic acid plus cholesterol diets for 8 weeks

Lipoprotein fraction	Diet	Protein	TL	PL	TC
			(mg in 100 ml serum)[a]		
VLDL	Control	1.0 ± 0.2	5.8 ± 3.1	1.2 ± 0.5	2.6 ± 0.8
	C	86.6 ± 31.5	258.1 ± 136.9	49.1 ± 32.5	251.8 ± 121.2
	CO	28.0 ± 23.8	92.7 ± 81.5	17.8 ± 16.2	71.1 ± 68.0
LDL	Control	27.1 ± 1.5	36.8 ± 8.6	13.7 ± 4.2	25.6 ± 6.7
	C	59.9 ± 5.2	157.7 ± 47.1	38.3 ± 8.8	152.3 ± 41.3
	CO	21.9 ± 5.5	57.8 ± 18.8	14.6 ± 3.8	44.1 ± 13.4
HDL	Control	202.1 ± 21.3	136.3 ± 30.8	98.3 ± 24.4	65.5 ± 16.4
	C	167.2 ± 21.5	111.5 ± 12.8	41.4 ± 5.4	77.1 ± 13.9
	CO	127.9 ± 13.8	75.7 ± 15.4	39.3 ± 10.5	45.0 ± 7.0

[a]Each value is the mean ± S.D. of the respective fraction from 4 chickens.

and group O birds and intermediate in the group CO chickens (Tables 2 and 3). Much of the differences observed in the LDL levels were probably due to the difficulty encountered in separating the often yellow, gel-like VLDL fraction from the LDL fraction after ultracentrifugation (Kruski & Narayan, 1972*a*). The composition of serum HDL fraction of the control was similar to that of group O fed birds (Table 2). The HDL protein, and PL levels decreased 40 and 70% (both $P = 0.01$) for the group C, and 47 and 62% (both $P = 0.01$) for the group CO birds respectively during the 4 week feeding period compared to the controls. Similar decreases of 17 ($P = 0.01$) and 58% ($P < 0.001$) for the group C, and 37 and 60% (both $P < 0.001$) for group CO animals were noted in the protein, and PL levels, respectively after 8 weeks on the diets (Table 3).

After labelled, leucine was administered to birds fed the 4 diets, the most striking difference in incorporation into the different serum fractions occurred in

Table 4. L-Leucine UL-^{14}C incorporation *in vivo* into serum lipoproteins of chickens fed cholesterol, orotic acid and cholesterol plus orotic acid diets[a]

Diet	Diet (weeks)	Total activity[b] VLDL	LDL	HDL	BF[c]
Control	4	0	7.2 ± 1.0	36.3 ± 6.6	159.8 ± 9.8
	8	0	6.6 ± 1.5	28.0 ± 6.7	123.4 ± 17.5
1% Orotic acid	4	0	10.5 ± 2.1	46.1 ± 16.1	202.5 ± 51.1
1% Cholesterol	4	11.5 ± 2.8	12.1 ± 5.0	26.7 ± 12.0	196.5 ± 72.8
	8	9.7 ± 1.5	7.9 ± 2.0	28.0 ± 7.7	139.7 ± 34.5
1% Cholesterol + 1% Orotic acid	4	4.2 ± 2.4	12.5 ± 2.8	25.1 ± 6.7	178.5 ± 63.1
	8	3.7 ± 2.6	4.1 ± 1.4	20.5 ± 6.2	94.3 ± 27.8

[a]Radioactive label was administered by intraperitoneal injection to chickens fasted for 12–18 hr. The animals were sacrificed 4 hr after injection. Two chickens per diet were used for the 4 week diet while 4 chickens per diet were used for the 8 week diet.

[b]Total activity = dis/min of radioactive leucine incorporated in 100 ml of chicken serum × 10^4 ± S.D.

[c]BF = bottom fraction, serum proteins remaining after ultracentrifugal removal of lipoproteins.

the VLDL fraction (Table 4). No incorporation was found in animals from control and group O whereas incorporation did occur in the VLDL fraction of group C and group CO birds. The VLDL fraction from the group CO birds had about 37% ($P < 0.01$) of the total radioactivity found in the group C animals for both the 4- and 8-week feeding periods.

DISCUSSION

The chicken responds to a 1% orotic acid diet in a different manner than does the rat. Unlike the rat (Windmueller & Levy, 1967; Windmueller, 1964; Windmueller *et al.*, 1970), this orotic acid diet caused little change in either liver or serum TC levels as well as serum LDL concentrations compared to control animals (Tables 1–3), even though orotic acid is known to affect liver purine to pyrimidine ratios in chickens (Bloomfield *et al.*, 1969). Similarly, like the control animals, no labelled leucine was found in the VLDL fraction. Incorporation of the label into the other lipoprotein fractions was similar for the birds on the 2 diets. The absence of orotic acid-induced fatty livers in chickens seen here is in agreement with the findings of Bloomfield *et al.* (1969). Further, it may denote a species specificity since similar findings have been recently reported for the mouse (Furuno *et al.* 1975).

The VLDL and liver TC levels, as well as the amount of incorporation of label in VLDL, were all about 60% less in the group CO as compared to the group C birds. Previous results have shown that there is a direct relationship between VLDL and liver TC levels in chickens on a cholesterol diet (Kruski & Narayan, 1972*a*). Although a decrease in HDL protein and PL levels are known to occur in cholesterol-fed, as compared to normal chickens (Kruski & Narayan, 1972*a*; Jones & Dobrilovic, 1969; Leveille & Sauberlich, 1963), the almost identical decrease in the components when orotic acid was added to the cholesterol diet was unexpected (Tables 2 and 3). Since the group CO fed birds had lower VLDL and liver TC levels than the group C animals, it was expected to have a less pronounced effect on the HDL protein and PL levels than the group C diet. The inverse relationship between VLDL and HDL protein and PL levels may be explained by a direct transfer of these components from HDL to VLDL when a cholesterol diet is fed. A recent report indicates that rooster HDL has several low mol. wt polypeptides, which may be common with VLDL (Kruski & Scanu, 1975).

Since orotic acid (group O) does not alter the serum lipoprotein pattern (especially serum LDL levels) or composition in the chicken, it apparently does not inhibit hepatic lipoprotein synthesis, unlike in the rat (Windmueller & Levy, 1967; Windmueller *et al.*, 1970). Nonetheless, the lower VLDL and liver TC levels in the group *CO* as compared to group C birds could be due to a decreased hepatic lipoprotein synthesis. This is suggested by the lower total activity found in the VLDL of the group CO compared to group C animals, 4 hr after injection of labelled leucine. The possibility that orotic acid may interfere with dietary absorption or transport can not be determined by the present results.

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