Lipoprotein Hydrolysis and Fat Accumulation in Chicken Adipose Tissues Are Reduced by Chronic Administration of Lipoprotein Lipase Monoclonal Antibodies

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ABSTRACT The lipoprotein lipase (LPL) catalyzed hydrolysis of plasma lipoproteins is a rate-limiting step in the lipid transport into peripheral tissues. The aim of the present study was to isolate monoclonal antibodies against chicken adipose LPL and to investigate whether chronic infusion of the LPL monoclonal antibodies inhibits adipose LPL activity and consequently reduces fat accumulation in broiler chickens. The LPL catalyzed very low density lipoprotein (VLDL) hydrolysis was completely inhibited by the addition of 100 µg/mL of monoclonal antibodies (CLP10, CLP14, CLP16) in the in vitro incubation with plasma VLDL and LPL. A single injection of CLP10 and CLP16 into chickens fed or starved for 24 h elevated plasma triacylglycerol concentrations for 24 h, whereas that of CLP14 was ineffective. Intravenous injection every other day and continuous infusion by osmotic minipump with CLP16 maintained higher plasma triacylglycerol concentration for 5 d than that of the control group and extensively reduced LPL activity in adipose tissues and abdominal fat pad weight. Lipoprotein lipase mRNA and protein levels in adipose tissue were not modified by chronic administration of anti-LPL antibody. The results indicate that chronic administration of anti-LPL antibodies is effective in retarding fatness in broiler chickens, and the antibodies are a proper subject for studies of lipoprotein metabolism.

(Key words: lipoprotein lipase, monoclonal antibody, chronic administration, lipoprotein metabolism, fat accumulation)

INTRODUCTION

The lipogenic activity in liver is much greater than that in adipose tissues in chickens, and most of the fat accumulation in the adipose tissues may be accounted for by incorporation of triacylglycerols from plasma lipoproteins that are either synthesized in the liver or provided from dietary fats (Griffin and Hermier, 1988). In lipoprotein metabolism, lipoprotein lipase (LPL) catalyzed hydrolysis of tri- and di-acylglycerols in peripheral tissues is a rate-limiting step; absence or low activity of the LPL causes marked lipemia and triglyceridemia (Bensadoun, 1991).

Bensadoun and Kompiang (1979) observed a linear increase of plasma triacylglycerol concentration for 60 min following an intravenous injection of anti-LPL antiserum in roosters. Gershenwald et al. (1985) reported that the monoclonal antibody to avian LPL (CAL 1-11) was a highly specific inhibitor of LPL in vitro. It is, therefore, likely that LPL plays a crucial role for fat accumulation in adipose tissues and thereby the inhibition of LPL catalyzed hydrolysis of triacylglycerol-rich lipoproteins reduces the fat accumulation in chickens. However, in order to demonstrate the critical inclusion of LPL in the fat accumulation in chickens, it might be essential to provide evidence of whether chronic, but not temporary, inhibition of LPL reduces the fat accumulation. We anticipated, therefore, that chronic infusion of a highly specific inhibitor, particularly the monoclonal antibody against chicken adipose tissue LPL, into chickens might provide information on this point.

In the present work, we isolated monoclonal antibodies against chicken adipose LPL and investigated whether chronic infusion of the LPL monoclonal antibodies inhibits its adipose LPL activity and consequently reduces fat accumulation in broiler chickens.

MATERIALS AND METHODS

Animals

Male broiler chickens (Ross strain)2 were housed in an electrically heated battery brooder and fed a commercial

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Abbreviation Key: FFA = free fatty acid; LPL = lipoprotein lipase; VLDL = very low density lipoprotein.
broiler starter diet (CP 22%, crude fat 4%, 3,200 kcal/kg ME diet). The chickens in all experiments were housed individually in wire cages in a room with controlled temperature (25 ± 3°C).

**Purification of Chicken Lipoprotein Lipase**

Adipose LPL from chickens fed on a commercial broiler starter diet was purified by a procedure with three chromatography steps on Heparin-Sepharose 4B, Hydroxyapatite and Con A-Sepharose 4B as described previously (Sato et al., 1997). The specific activity of purified LPL was 64.87 U/mg protein.

**Preparation of Chicken Very Low Density Lipoprotein**

Blood was collected from a wing vein with heparin, as anticoagulant, and centrifuged for 15 min at 1,500 × g. Plasma lipoproteins, very low density lipoproteins (VLDL) (d = 0.96 to 1.006 g/mL), were prepared by the method of Lindgren (1975) using ultracentrifugation performed KONTRON ultracentrifuges3 with a TFT65.13 rotor.

**Preparation of Monoclonal Antibodies**

The production procedure of monoclonal antibodies against chicken LPL reported by Gershenwald et al. (1983) was employed with slight modifications. Eight-week-old Balb/c mice were injected in the abdominal cavity with was employed with slight modi

...rotor.

**(NH4)2SO2 at pH 7.0 and Protein A-Sepharose column 6 fi...**

immunosorbent assay. Monoclonal antibodies were purified to bind immobilized LPL in an enzyme-linked immunosorbent assay. Monoclonal antibodies were included in the incubation medium to give a final concentration of 0, 10, 20 and 100 µg/mL of monoclonal antibody followed by anti-mouse IgG with alkaliphosphatase conjugate. After rinsing five times in 20 mmol/L Tris-HCl (pH 7.5), 50 mM NaCl and 0.3% Tween 20, the strips were incubated in a substrate solution (NBT/BCIP-stable mix) for 5 to 10 min. The LPL proteins were determined semiquantitatively by densitometer tracing.

**Western Blot Analysis**

To analyze the RNA of LPL in chicken adipose tissue, Northern blots were hybridized with 32P-labeled probe primed with random hexanucleotides (Feinberg and Vogelstein, 1983). The probes used to analyze the Northern blots included the chicken, Gallus gallus, LPL cDNA extended from nucleotides 1 to 807 (Cooper et al., 1989), synthesized from chicken adipose tissue mRNAs by reverse transcriptase-polymerase chain reaction following a check of the LPL cDNA sequence. After a high stringency wash (0.1 × SSC/0.1% SDS at 60°C) blots were exposed to Kodak XAR-5 film with intensifying screen for 12 h at −70°C. The films were scanned with an image analyzer.

**Northern Blot Analysis**

In Experiment 1, in vitro incubations with plasma VLDL and purified LPL prepared from chicken adipose tissue were undertaken to demonstrate the effects of monoclonal antibodies on VLDL hydrolysis. Monoclonal antibodies were included in the incubation medium to give concentrations of 0, 10, 20 and 100 µg/mL. The purified chicken LPL (1 µg/mL) in 0.1 mL was pre-incubated with specified concentrations of anti-LPL monoclonal antibodies in 0.1 mL for 30 min at 4°C. Then 0.2 mL of chicken VLDL (120 µg/mL in final triglyceride concentration) was added with 50 mM Tris-HCl buffer, pH 8.6, containing 0.2 mL of fat-free bovine serum albumin solution (40 mg/mL) to give 0.6 mL in the final volume and incubated at 37°C for 60 min.

In Experiment 2, 5-wk-old male broiler chickens (body weight 1,203 ± 25 g) fed or starved for 24 h, were injected once with three independent monoclonal antibodies into the wing vein at 1 mg/kg body weight. The control group was intravenously injected with sterilized saline (0.15...
mol/L NaCl). Blood samples were collected before and 1, 2, 4, 6, 12, and 24 h after antibody injections.

In Experiment 3, 4-wk-old male broiler chickens (body weight 989 ± 13 g) were chronically infused with anti-LPL monoclonal antibody by intravenous injection into wing vein (1 mg/kg body weight) at 1:00 p.m. each day for 7 d or by continuous infusion (1 mg/kg body weight/d) using an osmotic minipump11 designed to deliver, in vivo, 1 µL/h at a constant rate over 7 d. The minipump, filled with antibody, was implanted transversely in chickens. The control group received sterilized saline with no antibody, by intravenous injection into wing vein every day for 7 d. Blood samples were obtained before and 1, 3, 5, and 7 d after antibody administration.

After the last blood sampling, the chickens were killed by cervical dislocation and abdominal adipose tissues were rapidly removed and chilled in ice-cold NaCl (0.157 mol/L) for determination of LPL activity or frozen in liquid nitrogen for Western and Northern blot analysis of LPL. The LPL activity was determined by in vitro incubation with crude LPL and triolein emulsion as described previously (Sato et al., 1997). Five chickens were assigned to each treatment in Experiments 2 and 3.

Other Assays

Protein content of enzyme and antibodies were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Triacylglycerol concentrations of plasma or VLDL were quantified by the method of Fletcher (1968).

Statistical Analysis

A SAS® applications package12 was used for statistical calculations. Group data for multiple comparisons were analyzed by ANOVA using a general linear models procedure followed by Duncan’s multiple range test. The level of significance used in all studies was P < 0.05.

RESULTS

Monoclonal Antibodies to Chicken Lipoprotein Lipase

Following the screening of 10,772 clones of hybridoma following the fusion of mouse spleen cells with myeloma, we obtained 24 positive clones to bind specifically with chicken LPL. In this report, the properties of three hybridomas, CLP10 (IgG3), CLP14 (IgG1), and CLP16 (IgG2a), are discussed. These hybridomas had high affinity to chicken LPL with no cross-reaction to rat LPL and were provided for the ligand of affinity chromatography for chicken LPL (data not shown).

Inhibition of Chicken Lipoprotein Lipase Catalytic Activity by Monoclonal Antibodies In Vitro (Experiment 1)

The rate of VLDL hydrolysis in vitro by LPL declined gradually with an increase of monoclonal antibody (CLP10, CLP14, and CLP16) concentration, and complete inhibition of LPL catalytic activities occurred at a concentration of 100 µg/mL for every antibody (Table 1).

Changes in Plasma Triacylglycerol Concentration After Single Injection of Monoclonal Antibodies (Experiment 2)

In fed and starved chickens, plasma triacylglycerol concentration gradually rose after monoclonal antibody (CLP10 and CLP16) injections and attained a plateau at 6 h to reach approximately 3-fold compared with the control. A small increase of plasma triacylglycerol concentration was observed with injection of CLP14 (Figure 1). Thus, CLP16 was selected for subsequent chronic infusion studies because injection with CLP16 maintained a high plasma triacylglycerol concentration up to 24 h.

Effects of Chronic Infusion of Anti-Lipoprotein Lipase Monoclonal Antibody (Experiment 3)

The infusion of CLP16, by intravenous sequential injection or continuous infusion using osmotic minipump markedly elevated plasma triacylglycerol concentration for 3 d, followed by gradual decrease toward a concentration similar to the control at Day 7 (Figure 2). The LPL activity in chickens that received intravenous injection for 7 d or continuous infusion were significantly lower than that in control chickens (Figure 3a). However, the expression of LPL protein and mRNA were not different between control- and antibody-infused groups (Figure 3b,c). In chickens chronically infused with LPL antibody, adipose fat pad weight was significantly lower than that of the controls, whereas body weight decreased slightly (Table 2).

### Table 1. Effect of anti-lipoprotein lipase (LPL) monoclonal antibodies on lipoprotein hydrolysis in vitro by chicken LPL

<table>
<thead>
<tr>
<th>Antibody</th>
<th>10 µg/mL</th>
<th>20 µg/mL</th>
<th>100 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>CLP10</td>
<td>25.9 ± 2.5a</td>
<td>13.6 ± 1.5b</td>
<td>1.6 ± 1.0c</td>
</tr>
<tr>
<td>CLP14</td>
<td>27.8 ± 2.4a</td>
<td>16.3 ± 1.1b</td>
<td>1.8 ± 1.2a</td>
</tr>
<tr>
<td>CLP16</td>
<td>32.5 ± 1.3a</td>
<td>23.4 ± 2.4ab</td>
<td>2.0 ± 1.5a</td>
</tr>
</tbody>
</table>

*Values with a different superscript within a row are different (P < 0.05).

1Lipoprotein hydrolysis was expressed as a percentage of lipoprotein hydrolysis with no addition of antibody and as mean ± SD (n = 4).
FIGURE 1. Changes in plasma triacylglycerol concentration after a single injection of lipoprotein lipase (LPL) monoclonal antibodies, CLP10 (○), CLP14 (△), and CLP16 (■), at a concentration of 1 mg/mL in fed chickens (A) and chickens starved for 24 h (B). The control group (●) was injected with sterilized saline (0.15 M NaCl). Results are expressed as mean ± SE (n = 5).

DISCUSSION

The present data showed the preparation of monoclonal antibodies of chicken adipose tissue LPL, which potentially inhibited LPL activity in both in vitro and in vivo experiments, and the use of the antibodies were of use in understanding the crucial role of LPL in lipid, particularly lipoprotein, metabolism. Three monoclonal antibodies, CLP10, CLP14, and CLP16, raised in the present study, specifically inhibited chicken LPL-catalyzed VLDL hydrolysis in vitro. In a single injection of antibodies to fed and starved birds, CLP10 and CLP16 augmented plasma triacylglycerol concentration and thereby caused marked lipemia, whereas CLP14 was not effective in inducing lipemia. These results suggest that CLP14 has a lower half-life in plasma, or it recognizes epitopes on other proteins and is competitively absorbed out of the circulation before interacting with LPL.

Chronic administration of CLP16 into chickens sustained high plasma triacylglycerol concentration for 3 d regardless of the infusion procedure; i.e. injection every day or continuous infusion by osmotic minipump. These results not only substantiated the previous finding by Bensadoun and Kompiang (1979) that an intravenous single injection of anti-LPL antiserum increased plasma triacylglycerol concentration for 60 min, but also demonstrated that the triglyceridemia was at least sustained for 5 d by continuous administration of the antibody. It was further evidenced that abdominal fat pad weight and adipose LPL activity were markedly decreased by the continuous administration of anti-LPL antibody for 7 d.

The reduction by half of abdominal fat content (percentage body weight) by the infusion of CLP16 was associated with decrease in feed intake by 14% in the present study. Beane et al. (1979) reported that feeding restricted to 85% of ad libitum consumption by broiler chickens did not reduce abdominal fat content. In contrast, Arafa et al. (1983) showed that feed restriction by 16% for 10 d caused a 10% decrease in abdominal fat content with a 16% decrease in body weight. These findings suggest that although a feed intake decrease cannot be fully ruled out, the decrease in abdominal fat content in chickens infused with LPL antibody is accounted for, to a large extent, by a marked inhibition of adipose LPL activity. These findings suggest that persistent administration of anti-LPL monoclonal antibody is effective in modifying LPL activity and consequently manipulating fatness in broiler chickens.

Lipemia induced by continuous administration of anti-LPL antibody was no longer maintained on Day 7 of the experiment when the LPL activity in adipose tissue was significantly lower than that of control chickens. Furthermore, LPL mRNA and protein levels in adipose tissue were not modified by monoclonal antibody administra-
FIGURE 3. Lipoprotein lipase (LPL) activity, LPL mRNA expression, and LPL protein in adipose tissues prepared from chickens receiving the chronic infusion of LPL monoclonal antibody (CLP-16) for 7 d.

(a) LPL activity was determined at 37°C incubation with triolein emulsion. One unit of enzyme activity was defined as the amount that released 1 mmol free fatty acid/h. Results are expressed as means ± SD (n = 4).

(b) Northern blot analysis of chicken LPL mRNA. Two micrograms poly(A) RNA prepared from chicken adipose tissue were hybridized with the chicken LPL probe and exposed to Kodak XAR-5 film with intensifying screen for 12 h at −70°C. Results are expressed as means ± SD (n = 4).

(c) Western blot analysis of chicken LPL. LPL crude extracts prepared from chicken adipose tissue were separated by 12.5% SDS-PAGE in absence of reducing agents and transferred to PVDF membrane. Results are expressed as means ± SD (n = 4).

Control (sequential intravenous injection of sterilized saline); Injection (sequential intravenous injection at 1 mg/kg body weight per d); Continuous infusion (continuous infusion at 1 mg/kg body weight per d using osmotic pump).

A feasible explanation for these phenomena is currently lacking, but it is possible that chronic depression of adipose tissue LPL stimulates alternative mechanisms for lipoprotein transport from blood plasma to peripheral tissues. Bujo et al. (1994) reported that VLDL receptors were highly expressed in hen oocytes. Frykman et al. (1995) observed that adipose tissue mass was extensively decreased in the VLDL receptor knockout mice. Taken together, it may be inferred that chronic inhibition of LPL by the antibody administration may enhance the VLDL receptor expression in adipose tissues to compensate for the decrease of LPL-mediated lipoprotein transport.

One might expect that depression of LPL-catalyzed lipoprotein transport by chronic administration of anti-LPL antibody increases the LPL mRNA level in adipose tissue to maintain lipid transport. Our results revealed, however, that the LPL mRNA expression and translation in adipose tissues is less sensitive to antibody manipulation in chickens. The LPL mRNA and protein in adipose tissue were not changed under the marked lipemia induced by CLP16 administration in this experiment. Chicken LPL mRNA may be insensitive to plasma concentration of VLDL as the substrate of LPL. Hermier et al. (1984) noted that chicken LPL activity is less responsive to nutritional manipulation as compared with mammalian LPL.

The plasma VLDL concentration of fully fed broiler chickens is positively correlated with the body fat content.

### Table 2. Influence of chronic infusion of anti-lipoprotein lipase (LPL) monoclonal antibody for 7 d on body weight gain, feed intake, and adipose fat pad weight in 28-d-old broiler chickens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight gain (g/7 d)</th>
<th>Feed intake (g/7 d)</th>
<th>Adipose fat pad weight (g/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>475 ± 17</td>
<td>751 ± 21</td>
<td>12.8 ± 3.3</td>
</tr>
<tr>
<td>Injection</td>
<td>458 ± 32</td>
<td>654 ± 23</td>
<td>6.2 ± 2.4</td>
</tr>
<tr>
<td>Continuous infusion</td>
<td>448 ± 26</td>
<td>645 ± 35</td>
<td>6.6 ± 2.8</td>
</tr>
</tbody>
</table>

*Values with different superscripts within a column are different (P < 0.05).

1Results are expressed as means ± SD (n = 5).

2Initial body weight was 989 ± 13 g.

3Control (sequential intravenous injection of sterilized saline); injection (sequential intravenous injection at 1 mg/kg body weight per d); continuous infusion (continuous infusion at 1 mg/kg body weight per d using osmotic pump).
or abdominal fat content (Griffin and Hermier, 1988). In using plasma VLDL concentration as a selection criteria, Whitehead and Griffin (1986) and Griffin et al. (1991) developed divergent lines of lean and fat broilers. On the other hand, Guo et al. (1988) showed that total LPL activity in abdominal fat tissues was significantly correlated with fat pad weight. In view of the foregoing, our findings show that not only is plasma VLDL concentration reduced but also LPL activity is inhibited by administration of LPL antibody and is an effective means of reducing the fatness of broilers.

In conclusion, monoclonal antibodies against adipose tissue LPL inhibit the VLDL hydrolysis by LPL in vitro and in vivo, and the chronic infusion of LPL monoclonal antibodies effectively manipulates fatness in broiler chickens.

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