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Relationships of Plasma and Very Low Density Lipoprotein Lipids and Subfractions with Abdominal Fat in Chickens

Teck Chwen Loh*, Bee Koon Tan, Hooi Ling Foo[1], Abdullah Norhani[2] and Idrus Zulkifli

Department of Animal Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT : A study was conducted to determine the relationships between triacylglycerol (TAG) of plasma, very low density lipoprotein (VLDL) and fat deposition in two different breeds of chickens. The VLDL apolipoproteins of both breeds were also characterised. The breeds used were crossbred village chicken (AK) (Sasso crossed) and commercial broiler (CB) (*Avian*). They were housed in six pens with 30 female and 30 male birds of each breed per pen. Three male and three female birds from each pen were slaughtered and the blood was collected. The VLDL was isolated and sub-fractionated using Fast Protein Liquid Chromatography (FPLC). VLDL TAG of CB was significantly lower than AK. The particle size was negatively correlated with VLDL TAG and positively correlated with abdominal fat. Sub-fraction 2 contained more apo E that will enhance the lipolysis process of the VLDL TAG than sub-fraction 1. CB had a higher proportion of sub-fraction 2 than AK. The results showed that the proportion of sub-fraction 2 was negatively correlated with VLDL TAG concentration and positively correlated with abdominal fat. (**Key Words :** Very Low Density Lipoprotein, Broiler, Crossbred Village Chicken, Apolipoprotein)

INTRODUCTION

Excess body fat deposition in broiler is now the concern of both producers and consumers. The fat represents as a waste to consumers who are concerned about nutritional and health aspects of their food. High body fat deposition in broiler chickens may represent an economic loss to producers, as it is inefficient in terms of energy metabolism and overall feed utilisation (Gaya et al., 2005). Moreover, obesity in birds increases the incidence of reproductive failure and death due to heart failure (Zubair and Leeson, 1996).

Very low density lipoprotein (VLDL) is a heterogeneous lipid-protein macromolecule complex that circulate in plasma and lymph. It is a globular micelle like particles that consist of a non-polar core of triacylglycerol (TAG) and cholestryl ester (CE) coated with a layer of amphiphilic compounds of phospholipid (PL), free cholesterol (FC) and protein that is called apolipoprotein (apo) (Smith et al., 1978). Very low density lipoprotein is one of the major lipoproteins transporting TAG from the liver to extrahepatic tissues, such as the adipose tissue, heart and lung (Cryer, 1981). Very low density lipoprotein metabolism plays an important role in fattening of poultry. It involves the process of synthesis, secretion and catabolism intravascularly. These processes lead to lipid uptake and storage by adipose tissue (Hermier, 1997). About 70-80% of VLDL TAG secreted into circulation is taken up by adipose tissue (Griffin et al., 1992). Liver is the main organ responsible for *de novo* lipogenesis in chicken (Saadoun and Leclercq, 1987). Griffin et al. (1991) reported that body fat content was highly correlated with rate of secretion of plasma TAG-rich lipoproteins. The difference in rate of VLDL secretion between lines appears to be caused by differences in both rates of hepatic lipogenesis and in the proportions of fatty acids directed towards lipoprotein synthesis and oxidation (Griffin et al., 1991).

Apolipoprotein of VLDL in chicken plasma and serum is different from that in human. The information of human apolipoprotein is well established (Elovson et al., 1988; Evans et al., 1989; Young, 1990), however the exact role of individual avian apolipoprotein is not known. It has been reported that VLDL of commercial broiler and crossed village chicken can be purified and subfractionated using fast protein liquid chromatography (Tan et al., 2005). The

* Corresponding Author : Loh Teck Chwen. Tel: +603-8946 6899, Fax: +603-8943 2954, E-mail: tcloh@putra.upm.edu.my

[1] Department of Bioprocess Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

[2] Department of Biochemistry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

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characteristics of apolipoprotein were studied in the subfractions of VLDL, however the relationships between abdominal fat and apolipoprotein are not well documented. Thus, the objectives of the present study were to study the relationships between plasma TAG, VLDL TAG, VLDL apolipoprotein and fat deposition in two breeds of chicken.

MATERIALS AND METHODS

Animals and husbandry

Two breeds of chicken used in this study were crossbred village chicken (Sasso crossed) (AK) and commercial broiler (*Avian*) (CB). The CB is considered as fat line chicken and AK as lean line chicken. They were used in order to study their differences in VLDL metabolism. One hundred and eighty day-old females and 180 day-old males from each breed were used. Crossbred village chickens were purchased from Pusat Kemajuan Peladang Selangor (PKPS), Puchong and commercial broiler were purchased from Sin Mah, Melaka, respectively. The birds were housed in six floor pens (3.72 m^2) containing a deep litter of wood shavings with 30 female and 30 male birds of each breed per pen with 3 replicates per breed. They were fed a conventional starter diet, up to three weeks of age and a finisher diet until six weeks of age for commercial broiler and 12 weeks of age for village chicken. Six weeks and 12 weeks are the marketing age for CB and AK, respectively. The feed and water were given *ad libitum*. They were kept on a 24 hours photoperiod. Weekly feed intake and body weights were taken.

Sampling

All the birds were tagged individually. Blood samples were collected at every three week intervals. The birds were fasted for 18 hours prior to blood withdrawal in order to ensure portomicrons were cleared from the circulation (Bachorik, 1982; Hermier, 1997). Three birds from both sexes from each pen were slaughtered and the blood samples were collected into vacutainer tubes (Beckton Dickinson, UK) containing disodium EDTA as anticoagulant to give a final concentration of 1 mg/ml of blood. The blood sample was mixed by inverting the collection tube gently in order to avoid hemolysis. The total abdominal adipose tissues were collected, weighed and kept in the polystyrene box with ice and then kept under -70°C.

Preparation of plasma samples

The blood samples were pooled in a clean glass test tube and centrifuged at 1,500×g for 30 minutes under 4°C. Plasma was transferred to a clean storage test tube by using a Pasteur pipette equipped with a rubber bulb. The plasma was then kept under -20°C before VLDL separation and composition analysis.

Very low density lipoprotein separation

Very low density lipoprotein was separated from the plasma by using Fast Protein Liquid Chromatography (Äkta-FPLC) (Amersham Pharmacia Biotech, Sweden) as described by Tan et al. (2005). After FPLC separation, the fractions were freeze-dried for 18 hours and kept under -20°C for composition analysis and subfractioning.

Subfractionation of VLDL by heparin-sepharose affinity chromatography

The VLDL was dialysed with 0.05 M NaCl, 5 mM Tris and 0.02% NaN_3, pH 7.4. $MnCl_2$ were added to not more than 1 mg of VLDL protein to a final concentration of 0.025 M. Subfractionation was carried out by using Fast Protein Liquid Chromatography (Äkta-FPLC). The heparin-Sepharose column (Amersham Pharmacia Biotech, Sweden) was equilibrated with three column volume (CV) of Buffer A (0.05 M NaCl, 5 mM Tris, 25 mM $MnCl_2$ and 0.02% NaN_3, pH 7.4). A three CV of buffer A was passed through the system to wash out the unbound protein followed by five CV of Buffer B (0.2 M NaCl, 5 mM Tris and 0.02% NaN_3, pH 7.4) and four CV of Buffer C (2.8 M NaCl, 5 mM Tris and 0.02% NaN_3, pH 7.4). Then, the system was washed by three CV of Buffer D (0.5 M NaCl, 0.01 M glycine and 0.02% NaN_3, pH 5.5) and Buffer E (0.5 M NaCl, 0.01 M glycine and 0.02% NaN_3, pH 8.5), respectively. Fractions were collected at 0.5 ml/min and absorbance was monitored at 280 nm. The fractions were dialysed against 0.15 M NaCl, 0.01% Na_2EDTA, 0.02% NaN_3, pH 7.4 for 24 h. The collected fractions were freeze-dried and studied for their apolipoprotein profiles.

Chemical composition analysis

The chemical compositions of the whole plasma and VLDL were determined. Plasma protein concentration was determined by Biuret method (Gornall et al., 1949), whereas VLDL protein concentration by the method of Lowry et al. (1951), using diagnostic kits provided by Sigma Diagnostics, Sigma Chemical Co. Ltd., Poole, Dorset, UK. The TAG and cholesterol concentrations were determined by an enzymatic method, using diagnostic kits provided by Randox Laboratories Ltd., UK. Free cholesterol and PL concentrations were determined by using kits provided by Wako Pure Chemical Industries, Ltd., Japan. The CE concentration was calculated as the difference between total cholesterol and free cholesterol concentration. The particle size was calculated according to Fungwee et al. (1992) by calculating the ratio of the sum of PL and FC over the sum of TAG and CE.

Postheparin plasma LPL activity assay

Postheparin plasma LPL activity assays were carried out using a modified method of Nozaki et al. (1984). One female and male bird from each pen was injected with 0.5 ml of 100 IU heparin/kg body weight. After 15 minutes, the birds were slaughtered. The blood was collected into clean test tubes placed in a polystyrene ice box. Plasma was isolated by centrifuging at 1,500 g for 30 minutes. The free fatty acids were measured by using non-esterified fatty acid kit purchased from Roche, Germany at 546 nm.

Statistical analysis

The trial was set up in a randomised complete block design. Lipid parameters were analysed with breed, sex and their interactions as the main effects. Data were analysed by analysis of variance (ANOVA) using the general linear model (SAS, 1989). However, no significant difference ($p>0.05$) was found for the interactions of main effects. Relationships among adipose tissues deposited, lipid parameters and VLDL subfractions were analysed by Pearson's correlation coefficient using linear regression (Minitab, 1995). Male and female chickens were used in the analyses. A total of 24 birds from AK and CB were used to study the relationships.

RESULTS AND DISCUSSIONS

Table 1 shows the total abdominal fat, plasma and VLDL protein and lipid concentrations, proportion of VLDL subfraction 2 and calculated VLDL particle size of female and male birds at week three and week six. Both sexes showed no significant different ($p>0.05$) of their plasma and VLDL protein lipid concentrations. The interaction between breed and sex was not significant ($p>0.05$) for the analysis of plasma and VLDL protein and lipid concentrations. At week six, the females deposited significantly ($p<0.01$) more fat than that of the male birds. These results are consistent with those reported by Whitehead and Griffin (1982). They found that although female birds were fatter than males, there is negligible effect of sex on plasma VLDL TAG concentration.

Table 2 shows that at 6th week, the plasma protein and lipid concentrations of AK were significantly lower ($p<0.05$) than CB except TAG concentration. From the correlation analysis (Table 3), the abdominal fat in birds was not correlated with plasma TAG concentration. Due to this, plasma TAG may not be a good indicator to predict body fatness in chicken. Plasma TAG consisted of TAG from portomicrons, VLDL, low density lipoprotein (LDL) and high density lipoprotein (HDL). The VLDL TAG should be used to predict body fatness instead of total plasma TAG.

The result shows that the abdominal fat ($p<0.01$) of AK was significantly lower than CB at week three and week six. However, the AK had a higher ($p<0.01$) VLDL TAG concentration than that of CB at week six (Table 2). There

Table 1. Total abdominal fat, plasma and VLDL protein and lipid concentrations, proportion of VLDL subfraction 2 and calculated VLDL particle size of female and male birds at weeks 3 and 6

Parameters	Week 3		p	Week 6		p
	Female	Male		Female	Male	
Abdominal fat	9.53±3.70	10.11±2.56	NS	20.82±6.30	15.55±3.58	**
Plasma						
Protein	42.74±3.44	42.72±6.45	NS	59.63±4.58	59.63±5.09	NS
TAG	0.59±0.26	0.44±0.14	NS	0.55±0.25	0.51±0.24	NS
PL	1.28±0.60	1.28±0.71	NS	1.93±0.54	2.10±0.39	NS
Cholesterol	1.38±0.16	1.47±0.24	NS	1.29±0.22	1.42±0.19	NS
FC	0.17±0.05	0.19±0.04	NS	0.09±0.04	0.11±0.04	NS
VLDL						
Protein	1.59±0.21	1.57±0.26	NS	1.23±0.06	1.16±0.11	NS
TAG	209.71±52.57	193.50±56.04	NS	45.02±28.36	29.58±26.40	NS
PL	86.84±52.59	81.31±44.19	NS	120.41±34.85	123.60±29.88	NS
Cholesterol	146.48±44.66	159.10±36.77	NS	117.05±30.82	114.07±22.32	NS
FC	45.12±10.68	45.93±10.95	NS	27.43±5.80	26.63±10.03	NS
Subfraction 2	59.30±8.76	49.73±12.11	*	39.49±10.59	42.34±10.89	NS
Particle size[a]	0.44±0.16	0.43±0.14	NS	1.13±0.29	1.30±0.29	NS

NS, $p>0.05$; * $p<0.05$; ** $p<0.01$.

The results are presented as mean±SD. Plasma composition concentrations and VLDL protein concentration are in mg/ml plasma. VLDL lipid concentrations are in μg/ml plasma. Fat is in g/kg BW and proportion of subfraction 2 is in %.

[a] Particle size = (PL+FC/TAG+CE) (Fungwee et al., 1992).

Table 2. Total abdominal fat, plasma and VLDL protein and lipid concentrations, proportion of VLDL subfraction 2 and calculated VLDL particle size of AK and CB at weeks 3 and 6

Parameters	Week 3		p	Week 6		p
	AK	CB		AK	CB	
Abdominal fat	7.49±2.84	12.14±0.76	**	14.85±3.77	21.52±5.41	**
Plasma						
Protein	42.83±4.36	42.63±5.87	NS	57.60±4.79	61.67±3.86	*
TAG	0.59±0.14	0.44±0.25	NS	0.64±0.29	0.42±0.08	*
PL	1.74±0.60	0.82±0.20	NS	1.70±0.39	2.33±0.29	**
Cholesterol	1.37±0.22	1.47±0.19	NS	1.21±0.12	1.51±0.17	**
FC	0.18±0.04	0.18±0.05	NS	0.08±0.03	0.12±0.04	**
VLDL						
Protein	1.47±0.20	1.69±0.21	*	1.17±0.08	1.21±0.10	NS
TAG	208.97±63.55	194.24±43.47	NS	56.46±25.52	18.14±13.36	**
PL	59.47±37.45	108.67±44.86	**	119.49±34.55	124.51±30.10	NS
Cholesterol	158.10±51.16	147.47±27.48	NS	103.59±27.18	127.53±20.04	*
FC	48.35±9.07	42.69±11.60	NS	28.12±8.37	25.94±7.87	NS
Subfraction 2	51.36±8.24	57.67±13.56	NS	35.42±8.20	46.42±10.10	**
Particle size[a]	0.36±0.14	0.51±0.11	**	1.15±0.26	1.29±0.32	NS

NS, p>0.05; * p<0.05; ** p<0.01.

The results are presented as mean±SD. Plasma composition concentrations and VLDL protein concentration are in mg/ml plasma. VLDL lipid concentrations are in μg/ml plasma. Fat is in g/kg BW and proportion of subfraction 2 is in %.

[a] Particle size = (PL+FC/TAG+CE) (Fungwee et al., 1992).

is a significant negative correlation (p<0.05) between abdominal fat and VLDL TAG concentration (Table 3). The results suggest that the uptake of TAG by adipose tissue of AK was lower compared to CB. This can be further supported by the lower postheparin plasma LPL activity in AK than CB (p<0.05). The LPL activities of AK and CB were 4.60±1.70 mM/h and 7.41±3.46 mM/h, respectively. The lower LPL activity in AK could has also accounted for the lower fat deposition. Hence, less TAG would be hydrolysed and little fatty acid would be released and deposited as fat. The result is in agreement with Whitehead (1988), who indicated that lean birds were expected to have lower LPL activities.

According to Gómez-Coronado et al. (1993), LPL prefer to act on larger, TAG-rich particles. The VLDL particle size of CB was bigger than AK at week three (p<0.01). The result is consistent with those reported by Gómez-Coronado et al. (1993). Beside this, from the correlation analysis, the particle size was positively correlated (p<0.05) with abdominal fat (Table 3). This suggests that CB with bigger particle size may lead to higher rates of LPL activity and higher hydrolysis of VLDL and more fat was deposited. This can be further supported by the correlation analysis between VLDL TAG concentration and particle size (Table 4). The VLDL TAG concentration of AK and CB was negatively correlated (p<0.05) with the particle size.

VLDL has been subfractioned by heparin-Sepharose affinity chromatography into four subfractions in rats by Gómez-Coronado et al. (1993), into two subfractions by Evans et al. (1989), Huff and Telford (1984), Nestel et al. (1983) and Trezzi et al. (1983) in humans. The VLDL is subfractioned according to the affinity of apo E to heparin. The second fraction contains more apo E than the first fraction and is retained longer in the column (Nestel et al., 1983; Trezzi et al., 1983; Huff and Telford, 1984; Evans et

Table 3. Relationships between abdominal fat and plasma TAG, VLDL TAG, VLDL particle size and proportion of subfraction 2 for AK and CB

Week	Parameters	R	p
3	Plasma TAG	-0.138	NS
	VLDL TAG	-0.499	*
	Particle size	0.535	**
	Subfraction 2	0.261	NS
6	Plasma TAG	-0.227	NS
	VLDL TAG	-0.451	*
	Particle size	0.199	NS
	Subfraction 2	0.344	NS

n = 24; NS, p>0.05; * p<0.05; ** p<0.01.

Table 4. Relationship between VLDL TAG concentration and VLDL particle size for AK and CB

Week	R	p
3	-0.422	*
6	-0.566	**

n = 24; * p<0.05; ** p<0.01.

Table 5. Relative proportion (%) of apolipoprotein present in subfraction 1 and 2 of AK and CB

Apo	Subfraction 1		Subfraction 2	
	AIV	E	AIV	E
AK	57.4±8.84	42.7±8.84	50.8±0.64	49.3±0.64
CB	52.8±1.84	47.2±1.84	49.8±0.71	50.2±0.71
p	NS	NS	NS	NS

NS, p>0.05. The results are presented as mean±SD.
Percentage was determined by using Alphalmager™ 1220 Documentation and Analysis System.

al., 1989; Wright et al., 1995; Loh et al., 2001). Table 5 shows the relative proportions of apo IV and apo E present in subfractions 1 and 2 of AK and CB. The proportion of apo E in subfraction 2 was numerically higher than subfraction 1. The apo E was believed to enhance the lipolysis process in human (Clark and Quarfordt, 1985). The apo E-poor subfraction or subfraction 1 contained higher TAG than subfraction 2 (Huff and Telford, 1984; Evans et al., 1989) and metabolised slower than subfraction 2 (Huff and Telford, 1984). Slow catabolism of subfraction 1 is due to the fact that it must first be converted to subfraction 2. The possibility remains that subfraction 1 is the precursor of subfraction 2, however; these two subfractions may be independently synthesised and catabolised (Huff and Telford, 1984).

The CB showed a higher proportion of subfraction 2 than AK at week six (Table 2). The results suggest that CB was having more apo E that lead to catabolism faster than AK. These results were further supported by the data shown in Table 5 that CB had more apo E than that of AK. Hence, more fatty acids were released for fat deposition. These findings were supported by the correlation analysis between abdominal fat and proportion of subfraction 2 (Table 3). The abdominal fat showed a positive correlation with proportion of subfraction 2. Gómez-Coronado et al. (1993) reported that the subfraction richest in apo E showed the lowest degree of TAG hydrolysis by LPL and lowest ability to deliver fatty acids to adipose tissue in rat. However, the actual role of apo E in VLDL metabolism in fat and lean lines chicken is not well understood.

In conclusions, CB had a bigger VLDL particle size and higher proportion of subfraction2 than AK. All these lead to higher rate of VLDL TAG hydrolysis and more fat was deposited.

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