



## Estimation of Lipoprotein-lipase Activity (LPL) and Other Biochemical Changes in Two Breeds of Overfeeding Geese\*

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**ABSTRACT :** This study aimed to examine the effect of overfeeding on biochemical parameters and lipoprotein-lipase (LPL) mRNA expression in different tissues associated with hepatic lipogenesis in Sichuan white and Landes geese. Fifty healthy male Sichuan white geese and fifty male Landes geese (*Cygnus atratus*) were hatched on the same day under the same feeding conditions and were selected as experimental animals. After overfeeding for 14 days (from 14 weeks to 16 weeks) and then slaughtering, the biochemical changes of hepatic lipogenesis were evaluated. Results showed that i) in Landes geese, the plasma concentration of glucose was higher ( $p < 0.001$ ), while plasma concentrations of insulin and VLDL were both lower ( $p < 0.01$ ); ii) the LPL mRNA level in pectoralis muscle and leg muscle of the overfed groups in both breeds was higher ( $p < 0.05$ ) than in the control groups; iii) in Sichuan white geese, the proportion of fatty liver weight was positively correlated with plasma triacylglycerols (TG) ( $p < 0.05$ ) and VLDL concentrations ( $p < 0.05$ ), while these correlations were not significant in Landes geese; and iv) the activity of LPL had significant positive correlation with the proportions of lipids in subcutaneous adipose tissue and abdominal adipose tissue in Sichuan white geese, while in Landes geese the correlation was negative ( $p < 0.05$ ) with proportions of lipids in the liver, LPL activity had a significant positive correlation with the proportions of lipids in subcutaneous adipose tissue. These results suggest that the Landes geese have a better ability to use the massive amount of ingested food and to store lipids preferentially in the liver, but the Sichuan white geese have a relatively lower ability to use energetic nutrients and lipid storage is more efficient in the adipose tissues. (**Key Words :** Fatty Liver, Goose, LPL Activity, LPL Expression, Overfeeding)

### INTRODUCTION

Fatty liver occurs in birds when the increase in lipogenesis exceeds the capacity for synthesis and secretion of lipoproteins (Hermier, 1997). So birds, especially some wild waterfowl, are more likely to show non-pathological hepatic steatosis as a consequence of energy storage before migration (Pilo and George, 1983). Moreover, at present, fatty liver (foie gras) is famous with a delicate texture and delicious flavor, many farmers take advantage of this natural susceptibility to steatosis for the production of fatty liver. However, the adaptive mechanisms inducing higher synthesis of hepatic lipogenic enzymes or fatty acids in response to carbohydrate-rich diets are unclear and remain to be determined in avian species.

Interestingly, the ability of birds to form fatty liver in response to overfeeding depends not only on the species, but also on the breed of Palmipedes (Hermier et al., 1991; Poujardieu et al., 1994; Hermier et al., 1999). Uptake of plasma lipids into extra-hepatic tissues is mediated by lipoprotein-lipase (LPL), which hydrolyses the portomicron and low density lipoprotein (VLDL) triacylglycerols. LPL is a glycoprotein enzyme that is produced in several tissues in mammals, such as adipose tissue, skeletal muscle, heart, macrophages and lactating mammary gland, but not in the adult liver (Hoenig et al., 2006; Albalat et al., 2007). Once LPL is synthesized, the enzyme is secreted and transferred to the luminal surface of capillary endothelial cells where it performs its function (Camps et al., 1990; Auwerx et al., 1992). For example, in the chicken, an increase in LPL activity may account for dramatic peripheral fat deposition (Whitehead and Griffin, 1982; Griffin et al., 1987). In addition, Sato et al. (1999) demonstrated that the chronic infusion of LPL monoclonal antibodies effectively manipulates fatness in broiler chickens. In overfed Landes geese, LPL activity is negatively correlated with fatty liver

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weight (Davail et al., 2000). In ducks, Pekin duck showed a higher LPL activity than Mule duck (Davail et al., 2003a) or Muscovy duck (Davail et al., 2003b), and the Mule duck and Muscovy duck showed a higher susceptibility to hepatic steatosis (Guy et al., 1999).

All these data suggest that the degree of fatty liver in waterfowl depends mainly, though not only, on the intensity of the hepatic lipogenesis, and also on the peripheral activity of LPL. Landes goose is a European goose breed and famous for a high capability of fatty liver production, and Sichuan white goose is a Chinese breed having a good capability for egg laying and meat production. The present study was designed to elucidate the correlation between LPL activities, expression of LPL in different tissues and measured plasma parameters including triacylglycerol (TG) concentrations, glucose and insulin in control and overfed geese of the Landes and Sichuan white breeds.

## MATERIALS AND METHODS

### Animals

Fifty male Sichuan white geese and fifty male Landes geese hatched on the same day, were grown under natural conditions of light and temperature at the experimental Station for Waterfowl Breeding of Sichuan Agricultural University. From 0 to 13 weeks, they were housed collectively and then were raised in separated pens during the overfeeding period. From 0 to 4 week of age, the geese had free access to a starting diet containing 2,900 kcal and 205 g protein/kg. From 4 to 13 weeks, they were given a growing diet in which daily intake was reduced to avoid excessive fatness. Because there was a difference in body weight between the Sichuan white geese and Landes geese, their amounts of food intake were different. In order to

obtain accurate results, the value of their daily food intake was calculated according to their body weight. Thus the daily amount of feed provided was 200 g in Sichuan white geese and 250 g in Landes geese between four and five weeks, 300 g in Sichuan white geese and 375 g in Landes geese between five and eight weeks, and 150 g in Sichuan white geese and 200 g in Landes geese between eight and thirteen weeks. At fourteen weeks of age, they were divided into two groups. The first group continued the control diet and was allowed to feed *ad libitum*, while the remaining geese were switched to an overfeeding diet containing two-thirds salted and boiled maize (3,370 kcal/kg, 90 g protein/kg, and 4.5 g fat/kg) with 0.4% waterfowl fat and one-third water added. For comparison between the body weights of the two breeds before overfeeding and after overfeeding, body weight data prior to overfeeding is shown in Table 1. Geese of the Sichuan white breed, having a lower capacity for overfeeding ingestion, were fed by the operator at the maximum of their ingestion potential. In total, the food intakes during the overfeeding period were 14,260 and 15,960 g for the Sichuan white and Landes geese, respectively. During the overfeeding period, birds were housed in individual cages. Animals had free access to water at all times. In the overfeeding room, the temperature was 15-18°C and relative humidity was 70-80%. The protocol for bird treatment was in accordance with the Canadian Council on Animal Care guidelines (1994).

### Experimental design

On the last day of the overfeeding, geese were fasted overnight (18 h) before experimentation. Plasma glucose and triacylglycerol were measured during the postprandial period of the first meal in 12 geese from the different genotypes. 5 min after the first overfeeding meal, the initial

**Table 1.** Influence of overfeeding on body composition

	Control group		Overfed group	
	Landes	Sichuan	Landes	Sichuan
Birth weight (g)	100.40±9.15***	90.96±5.46	103.52±11.73***	91.64±6.02
Body weight at 14 weeks (g)	4,656.57±329.79	3,855.00±252.12	4,632.14±279.66	3,907.20±269.62
Body weight at 16 weeks (g)	4,815.14±368.6***	4,133.33±318.49	6,051.33±514.75*** <sup>b</sup>	5,111.30±420.52 <sup>c</sup>
Liver weight (g)	90.57±16.08	83.00±11.86	519.33±124.31*** <sup>c</sup>	310.74±79.94 <sup>c</sup>
Liver weight (% BW)	1.89±0.31	2.01±0.26	8.60±2.10* <sup>c</sup>	6.10±1.60 <sup>c</sup>
Lipid proportion (% LW)	3.26±1.73*	1.88±0.58	49.06±7.31** <sup>c</sup>	38.47±7.16 <sup>c</sup>
TG content of lipid (%)	8.21±0.90**	5.32±0.47	94.07±0.99** <sup>c</sup>	90.92±2.34 <sup>c</sup>
Scat+skin (g)	812.86±26.98	607.00±27.25	1273.30±221.10* <sup>c</sup>	1110.40±189.00 <sup>c</sup>
Scat+skin (% BW)	16.85±2.03	14.62±1.70	21.00±3.80 <sup>a</sup>	21.60±3.70 <sup>a</sup>
Abdominal fat pad (g)	145.00±7.01	80.80±9.57	265.50±59.00 <sup>c</sup>	234.80±70.50 <sup>c</sup>
Abdominal fat pad (% BW)	3.01±0.49	1.94±0.62	4.40±1.00 <sup>a</sup>	4.50±1.40 <sup>b</sup>

BW = Body weight; LW = Liver weight; Scat = Subcutaneous adipose tissue; TG = Triglycerides.

\*. \*\*. \*\*\* Difference between Landes and Sichuan white geese at  $p < 0.05$ , 0.01 and 0.001, respectively.

<sup>a, b, c</sup> Effect of overfeeding at  $p < 0.05$ ,  $p < 0.01$  and 0.001, respectively.

5 ml of blood sampled from the wing vein was collected on EDTA (0.8 g/L) in a vacuum tube and kept at 2–4°C during the subsequent procedure; the subsequent blood samplings were performed 1, 2 and 4 h after the meal. Plasma was separated by centrifugation at 3,000 g for 10 min at 4°C and kept at -20°C until analysis for glucose concentration. Then, plasma insulin and LPL activity were measured 60 min after the first blood collection of the overfeeding period. Because LPL is anchored to the endothelial cell wall of the extrahepatic tissue by attachment to heparin or heparin-like components (Cryer, 1981; Pedersen et al., 1983), in order to measure activity of LPL in plasma the enzyme must be released into the general circulation by intravenous injection of heparin. Therefore, geese were intravenously injected with heparin (400 IU/kg BW) 60 min after the meal, then 5 min after heparin injection 3 ml blood samples were collected from the wing vein. Individual plasma samples were separated by centrifugation at 3,000 g for 10 min. Antibacterial agents (sodium azide 0.1 g/L) and a chelator of metal cations (EDTA 0.8 g/L) were added to plasma samples. Plasma samples were divided into two different groups, one was frozen at -20°C until further analyses, and the other was frozen at -80°C for LPL activity analyses.

At the end of the 13-day overfeeding period and after one night fasting, the fasted geese were killed in a slaughter house by exsanguination. The liver, abdominal adipose tissue and left filet were quickly removed and weighed, and a sample (approximately 20 g) was immediately taken from the ventromedial portion of the main lobe (right lobe) of each liver, and frozen at -80°C until analysis of enzymatic activity and mRNA level.

#### Liver and blood analyses

Livers were characterized for total lipid content and TG content. Total lipids were estimated after freeze-drying of 1 to 2 g of liver and extraction in a Soxhlet extractor in petroleum ether at 40 to 65°C. After weighing, liver lipid was extracted and preserved in a solution of chloroform-methanol (9:1, v/v) from another freeze-dried sample of 1 to 2 g of liver and stored at -20°C. Liver TG content was assayed by the acetylacetone method (Chen, 1999).

The whole plasma parameters such as triglycerides (TG), glucose, insulin and activity of lipoprotein lipase were determined using the corresponding kit as follows: plasma glucose was determined enzymatically using a Beckman autoanalyser and the corresponding kit (Kadish et al., 1968) and TG were quantified in whole plasma by colorimetric enzymatic methods (Fossati and Prencipe, 1982) using kits provided by the Shanghai Sangon Biological Engineering Technology and Service Co., Ltd.

Plasma insulin levels were determined by a radioimmunoassay with guinea pig-porcine insulin antibody using chicken insulin as the standard (Ruffier et al., 1998).

According to the procedure of Benson et al. (1975), post-heparin LPL activity was determined in plasma from fed animals. The method was as follows: 1 ml plasma was added to 1 ml of 0.1 M NH<sub>4</sub>Cl/NH<sub>4</sub>OH (pH 8.6) containing 3% bovine serum albumin and 15% Intralipid and preincubated at 37°C for 30 min with heat-inactivated (56°C, 10 min) geese serum. The fatty acids resulting from substrate hydrolysis after 60 min incubation at 37°C were extracted and determined colorimetrically. Lipoprotein lipase activity was expressed as μMol of palmitate released per ml per h.

In addition, VLDL were separated by ultracentrifugation at 100,000 g for 18 h at 10°C according to the procedure of Havel et al. (1955) and the concentration was determined as described previously (Fournier et al., 1997). Moreover, the density is commonly from 0.96 kg/L to 1.006 kg/L.

#### RNA isolation and cDNA synthesis

Total RNA was extracted from subcutaneous adipose tissue and the abdominal fat pad using the TRIzol reagent (Invitrogen) and treated with RNase-free DNase I (TakaRa Biotechnology Co. Ltd., Dalian, China) to remove contaminated genomic DNA. The first strand cDNA was synthesized using the ImProm-II Reverse Transcription System (TakaRa Biotechnology Co. Ltd., Dalian, China) according to the manufacturer's instructions. The reaction was performed in a volume of 10 μl containing 5×PrimerScript Buffer, 10 mM of each dNTPs, 40 U/μl RNase Inhibitor, 2.5 μM oligo-dT Primer. The reverse transcription was maintained at 30°C for 10 min, then 45°C for 25 min and ended with incubation at 95°C for 5 min. The cDNA product was stored at -20°C.

#### Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was used to measure the gene expression patterns of LPL mRNA in different tissues. Twelve geese including six Landes goose and six Sichuan White goose were used. Semi-quantitative RT-PCR was performed to determine the presence and relative level of *LPL* mRNA in various tissues, using a constitutively expressed gene, β-actin (Genbank No: M26111) (Forward: 5'-ACC CAC ACC GTG CCC ATC TAT G -3' and reverse 5'-GTG GCC ATC TCC TGC TCG AAG T -3'), as a positive control. RT-PCR was performed as described using an *LPL* gene (Genbank No: EF620913) specific primer forward: 5'-GGA CGG TGA CAG GAA TGT ATG A and reverse 5'-CAG CAG GAT CCA GAC CAG TAA. The constitutively expressed mRNA for β-actin was employed as a positive control in RT-PCR to normalize the levels of *LPL* mRNA in similar cDNA samples. PCR was performed in a 25 μl reaction volume containing 2.0 μl cDNA, 25 mM MgCl<sub>2</sub>, 10×Reaction Buffer, 2.5 mM dNTP Mix, 5 units of

**Table 2.** Plasma insulin, glucose, TG and VLDL measured 90 min after meal

Parameters	Control group		Overfed group	
	Landes geese	Sichuan White geese	Landes geese	Sichuan White geese
Plasma glucose (g/L)	9.76±0.41*	8.54±0.33	6.35±1.52***, a	2.76±1.31 <sup>b</sup>
Plasma insulin (μU/ml)	7.92±0.27**	6.56±0.35	10.28±0.31 <sup>a</sup>	11.53±0.28** <sup>b</sup>
TG (mmol/L)	0.35±0.22	0.60±0.27*	3.71±1.39 <sup>b</sup>	4.87±1.29 <sup>b</sup>
VLDL (g/L)	0.16±0.10	0.27±0.12*	1.70±0.64 <sup>b</sup>	2.20±0.59** <sup>b</sup>

TG = Triglycerides; VLDL = Low density lipoprotein.

\*\*\*\*\* Difference between Landes and Sichuan white geese at  $p < 0.05$ ,  $0.01$  and  $0.001$ , respectively.

<sup>a, b</sup> Difference between values at the first and the fourteenth day at  $p < 0.01$  and  $0.001$ , respectively.

Taq DNA polymerase and 10 μM of each primer. Amplification was carried out using the following procedure: initial denaturation at 94°C for 5 min; 30 cycles (24 cycles for β-actin) of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min; with a final elongation at 72°C for 10 min. After amplification, 2 μl of PCR product was analyzed by electrophoresis in 1% agarose gel, staining with ethidium bromide (EB) under an ultraviolet lamp, and then scanning through the Gel Imaging System.

### Statistical analysis

Data were analyzed by analysis of variance using the General Linear Model procedure of SAS6.12 (SAS Institute Inc., Cary, NC, 1997). The model included the effects of breed and time. Statistical differences between means were determined by ANOVA and the means were compared for significance by Newman-Keuls' test; the accepted  $p$ -value was 0.05. Correlations were determined by linear regression. In addition, the PCR semi-quantitative analysis used the Quantity One software (Bio-Rad, company). *LPL* gene expression levels were semi-quantified relatively to the expression of the *beta-actin* according to the formula as follows:

$$\text{Rel. Quantity} = B/A$$

Where Rel. Quantity is the relative quantity of the *LPL* in the sample compared with that in the control; B is the value of the target mRNA and A is the mRNA value of the internal control. Gene semi-quantity expression levels between different tissues from the group-related samples of the same breed were analyzed by one-way ANOVA. A  $p$ -value of  $< 0.05$  was considered statistically significant.

## RESULTS

### Body composition

Overfeeding in which the amount of food was proportional to animal body weight led to higher body weight gain in Landes geese (1,419.19±253.09 g) than in Sichuan white geese (1,204.10±150.90 g). The same

hierarchy can be seen in the weight of fatty liver and subcutaneous adipose tissue or abdominal fat pad from the two breeds (Table 1). However, since the body weights of the two strains were very different at the end of overfeeding, it was more informative to express the weight of organs as a percentage of the body weight. Under these conditions, Landes geese showed a massive liver steatosis (8.6% BW), that was moderate and identical in the other breed (6.1% BW). The proportion of subcutaneous adipose tissue was similar in Landes and Sichuan white geese (21.0% and 21.6% BW, respectively) and the abdominal adipose tissue was similar in Landes and Sichuan white geese (4.4% and 4.5% BW, respectively).

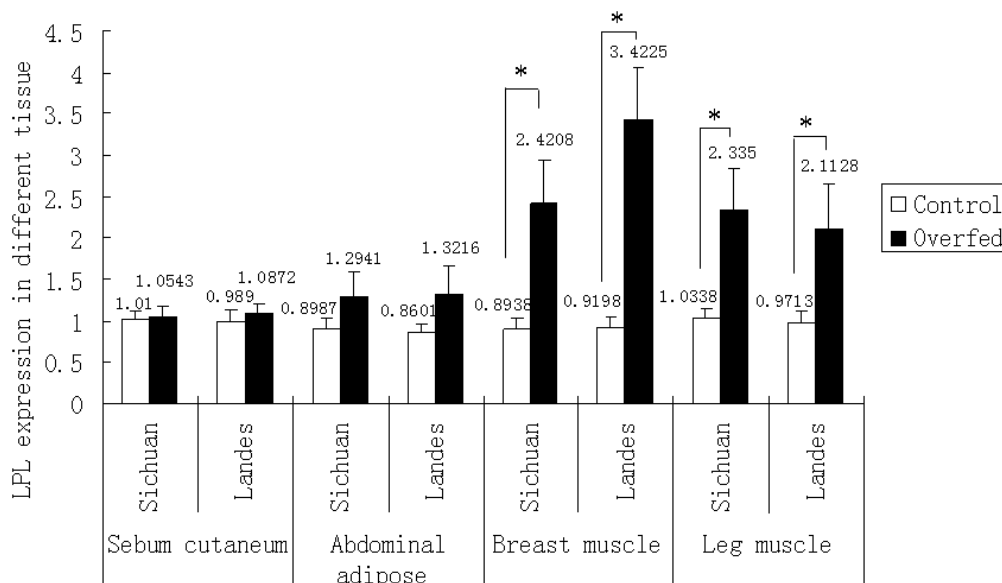
### Plasma parameters

Plasma glucose, insulin, VLDL and TG during the overfeeding period are reported in Table 2. From this data, we discovered that in the control group, plasma glucose and insulin in Landes geese were higher ( $p < 0.05$ ) than in Sichuan white geese, while the plasma concentrations of VLDL and TG were higher in Sichuan white geese than in Landes geese ( $p < 0.05$ ). In the overfed group, the changes of TG concentrations were not statistically significant in the two breeds; and the glucose level was higher in Landes geese ( $p < 0.001$ ), but the plasma concentrations of insulin and VLDL were both lower in Landes geese ( $p < 0.01$ ).

### Activities of LPL and LPL expression

Through our analysis, we found that in the overfed group, the activity of LPL was significantly higher in Sichuan white geese (11.40±3.06) than in Landes geese (7.61±2.13) while there was no difference between the two breeds in the control group (4.98±0.72 vs. 4.75±0.63). Compared with the control group, the activity of LPL in the two breeds was significantly higher in the overfed group ( $p < 0.001$ ).

*LPL* mRNA expression is shown in Figure 1. In the overfed group, the content of the *LPL* mRNA in abdominal adipose tissue, breast muscle and leg muscle was significantly higher than in the control group for both breeds ( $p < 0.05$ ), while there was no difference in the *LPL* mRNA levels of sebum cutaneum in either breed in the



**Figure 1.** LPL expression levels in various tissues in geese from different groups. \* indicates  $p < 0.05$ .

control group. In addition, in the overfed group the *LPL* mRNA level of breast muscle was higher in Landes geese, and the *LPL* mRNA levels of leg muscle was higher in Sichuan white geese. The *LPL* mRNA level in the sebum cutaneum and the abdominal adipose tissue showed no evident difference between the two breeds.

**Correlation analysis**

Correlations between body composition data and plasma metabolic substrates measured after the overfeeding period were calculated (Table 3). In Sichuan white geese, the proportion of fatty liver weight was positively correlated with plasma TG ( $r = 0.68, p < 0.05$ ) and VLDL concentrations ( $r = 0.55, p < 0.05$ ). These correlations were not significant in Landes geese. In the two breeds, the concentration of TG was positively correlated with plasma VLDL concentration (Landes geese:  $r = 0.99$ , Sichuan white geese:  $r = 0.98, p < 0.01$ ). No significant correlations were found in either breed between adipose tissues and plasma parameters measured in the overfed animals.

After overfeeding, LPL activity in Sichuan white geese was positively correlated with the proportions of subcutaneous adipose tissue ( $r = 0.78, p < 0.01$ ) and the proportions of abdominal adipose tissue ( $r = 0.52, p < 0.05$ ) and was negatively correlated with the plasma concentration of VLDL ( $r = -0.57, p < 0.05$ ), but had no evident correlation with the proportions of liver weight. However, the activity of LPL in Landes geese was negatively correlated with the proportions of lipids in liver weight ( $r = -0.54, p < 0.05$ ) and positively correlated with the proportions of lipids in subcutaneous adipose tissue ( $r = 0.56, p < 0.01$ ), but had no evident correlation with the proportions of lipids in abdominal adipose tissue and the plasma concentration of VLDL.

**DISCUSSION**

In birds, lipogenesis occurs almost exclusively in the liver (Saadoun and Leclercq, 1987; Mourot et al., 2000). Thus, to be stored in extrahepatic tissues (mainly

**Table 3.** Correlation between body composition and plasma VLDL, TG concentrations and Lipoprotein lipase activity after overfeeding

Landes	LPL	VLDL	TG	LWP	ScatP	AbdP	Sichuan	LPL	VLDL	TG	LWP	ScatP	AbdP
LPL	1	-0.32	-0.33	-0.54*	0.21	0.56*	LPL	1	-0.57*	-0.41	-0.36	0.52*	0.78**
VLDL		1	0.99**	-0.14	0.16	-0.28	VLDL		1	0.98**	0.55*	0.48	0.21
TG			1	0.26	-0.15	0.27	TG			1	0.68*	-0.48	-0.12
LWP				1	-0.18	-0.35	LWP				1	0.06	-0.12
ScatP					1	0.45	ScatP					1	0.50*
AbdP						1	AbdP						1

The number in the table expresses correlation coefficient \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; LWP = the proportion of liver weight in body weight; ScatP = the proportion of subcutaneous adipose tissue weight in body weight; AbdP = the proportion of Abdominal adipose tissue weight in body weight; TG = triglycerides; VLDL = Low density lipoprotein; LPL = Lipoprotein-lipase.

subcutaneous tissues and muscles), the newly synthesized triacylglycerols from dietary carbohydrates are incorporated into VLDLs which are secreted into the blood and hydrolyzed by LPL synthesized in peripheral tissues, allowing the uptake of fatty acids and thus fat storage. The plasma lipoprotein-triacylglycerols that are not hydrolyzed by LPL may return to the liver and contribute to hepatic steatosis after uptake by specific lipoprotein receptors and internalization as shown in mammals (Mulder et al., 1993). Consequently, different combinations of these four mechanisms (hepatic lipogenesis, hepatic lipoprotein secretion, LPL lipoprotein hydrolysis and hepatic lipoprotein reuptake) can modify fat storage repartitioning and explain the different overfeeding performances shown in two genotypes of duck (Hermier et al., 2003).

The hepatic lipogenesis and synthesis-secretion of VLDL does not fully explain the lipid storage distribution between the liver and the extrahepatic tissues: in particular, the adipose tissue distribution appears different in the two breeds of geese. Indeed, extrahepatic fattening necessitates the hydrolysis of VLDL triacylglycerols by LPL, which is produced by the peripheral tissues. In the control groups, LPL activity appeared to be similar in the two breeds of goose since: i) no difference was seen in the *in vitro* activity of post-heparin LPL; and ii) the triacylglycerol concentration of two breed geese was not different. Conversely, on the fourteenth day of the overfeeding period, this activity dramatically increased in both Landes ( $p < 0.01$  vs. the first day values) and Sichuan white geese. Considering the weight of tissues found after fourteen days of overfeeding and the possibility that hepatocytes have to uptake and internalize the lipoproteins not hydrolyzed by LPL (Mulder et al., 1993), it can be suggested that inhibition of LPL activity during overfeeding contributes significantly to increasing hepatic steatosis to the detriment of extrahepatic fattening as in Landes geese, while the opposite occurs in the Sichuan white geese.

In previous studies, some researchers have shown that the enzymatic activity of post-heparin LPL is higher in Pekin ducks than in Muscovy ducks (Davail et al., 2003b) or in Mule ducks (Davail et al., 2003a), especially at the end of the overfeeding period. In the present study, we found that neither LPL activity nor *LPL* mRNA levels showed any significant differences between control groups of the two breeds, so we speculated that i) the stability of *LPL* mRNA may be controlled by insulin, so the *LPL* mRNA level showed no difference between the control groups in the two breeds, or ii) the efficiency of *LPL* mRNA translation into protein was similar with the modulation of nutrition in Sichuan white geese and Landes geese, which indicates this may be associated with the level of some hormone, e.g., thyroid hormones. This is consistent with the conclusion

that hepatic lipogenesis and enzymatic activity of LPL are in part controlled by pancreatic hormones (review in Leclercq et al., 1984) and the insulin/glucagon ratio seems to be particularly important in the control of avian metabolism (Hazelwood, 1984). Conversely, after overfeeding, the LPL activity of Sichuan white geese is higher. So, it can be suggested that there was a significant difference between the two breeds in the efficiency of *LPL* mRNA translation or the modulation of LPL activity. In breast muscle, the *LPL* mRNA level in Landes was significantly higher, while in leg muscle, the *LPL* mRNA level in Sichuan white geese was higher.

The present study strongly suggests that: i) the growth rate of extrahepatic adipose tissues was greater in Sichuan white geese, while more lipids were accumulated in liver tissue in Landes geese; ii) Sichuan white geese had a higher plasma concentration of triacylglycerols, lipoproteins and insulin; however, Landes geese exhibited greater increase of plasma concentrations of TG, lipoproteins and insulin; iii) In the overfed group, the activity of LPL was significantly higher in Sichuan white geese than in Landes geese, while there was no difference between the two breeds in the control group. In addition, the mRNA level of *LPL* in pectoralis muscle and leg muscle in the overfed groups of both breeds was higher than in control groups. However, we also found that in the overfed group, the *LPL* mRNA level of pectoralis muscle was significantly higher in Landes than in Sichuan white geese, and the *LPL* mRNA level of leg muscle was significantly different between the two breeds, while the *LPL* mRNA level of the sebum cutaneum and the abdominal adipose tissue showed no evident difference in the two breeds; and iv) The correlations between LPL activity and the proportions of subcutaneous adipose tissue, abdominal adipose tissue and liver weight, and the plasma concentration of VLDL were different in the two breeds.

Overall, these data suggest that when food intake is corrected for body weight, Landes geese have a greater potential to use the massive amount of ingested food and to store lipids preferentially in the liver, while Sichuan white geese have a relatively lower utilization of energetic nutrients and lipid storage is more efficient in the adipose tissues.

## REFERENCES

- Albalat, A., A. Saera-Vila, E. Capilla, J. Gutierrez, J. Perez-Sanchez and I. Navarro. 2007. Insulin regulation of lipoprotein lipase (LPL) activity and expression in gilthead sea bream (*Sparus aurata*). *Comp. Biochem. Physiol. PartB*. 148:151-159.
- Auwerx, J., P. Leroy and K. Schoonjans. 1992. Lipoprotein lipase: recent contributions from molecular biology. *Crit. Rev. Clin. Lab. Sci.* 29:243-268.

- Berradi, H., G. Guy and N. Rideau. 2004. A glucokinase-like enzyme induced in mule duck livers by overfeeding. *Poult. Sci.* 83:161-168.
- Benson, J. D., V. Hearn, T. Boyd and A. Bensadoun. 1975. Triglyceride hydrolase of chicken and rat pre- and post-heparin plasma. Effects of fasting and comparison with adipose tissue lipoprotein lipase. *Int. J. Biochem.* 6:727-734.
- Cryer, A. 1981. Tissue lipoprotein-lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* 13:524-541.
- Camps, L., M. Reina, M. Llobera, S. Vilaró and T. Olivecrona. 1990. Lipoprotein lipase: cellular origin and functional distribution. *Am. J. Phys.* 258:C673-681.
- Chen, Z. Y. 1999. Handbook of clinic biochemistry and biochemistry experiment analysis. Beijing: Chinese Medicine Press. (in Chinese).
- Davail, S., G. Guy, J. M. André, D. Hermier and R. Hoo-Paris. 2000. Metabolism in two breeds of geese with moderate or large overfeeding induced liversteatosis. *Comp. Biochem. Physiol. Part A.* 126:91-99.
- Davail, S., N. Rideau, G. Guy, J. M. André and R. Hoo-Paris. 2003a. Pancreatic hormonal and metabolic responses in overfed ducks. *Horm. Metab. Res.* 35:439-443.
- Davail, S., N. Rideau, G. Guy, J. M. André, D. Hermier and R. Hoo-Paris. 2003b. Hormonal and metabolic responses to overfeeding in three genotypes of ducks. *Comp. Biochem. Physiol. Part A.* 134:707-715.
- Fossati, P. and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* 28:2077-2080.
- Fournier, E., R. Peresson, G. Guy and D. Hermier. 1997. Relationships between storage and secretion of hepatic lipids in two breeds of geese with different susceptibility to liver steatosis. *Poult. Sci.* 76:599-607.
- Guy, G., D. Hermier, S. Davail, M. Bely, J. M. André and R. Hoo-Paris. 1999. Meat production and force feeding ability of different types of ducks. 1<sup>st</sup> World Waterfowl Conference, Taichung (Taiwan). 1-4:462-468.
- Griffin, H. D., S. C. Butterwith and C. Goddard. 1987. Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chickens. *Br. Poult. Sci.* 28:197-206.
- Havel, R. J., H. A. Eder and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345-1353.
- Hazelwood, R. L. 1984. Pancreatic hormones, insulin/glucagon molar ratios, and somatostatin as determinants of avian carbohydrate metabolism. *J. Exp. Zool.* 232(3):647-652.
- Hermier, D., A. Saadoun, M. R. Salichon, N. Sellier, D. Rousselot-Pailley and M. J. Chapman. 1991. Plasma lipoproteins and liver lipids in two breeds of geese with different susceptibility to hepatic steatosis: Changes induced by development and force-feeding. *Lipids* 26:331-339.
- Hermier, D. 1997. Lipoprotein metabolism and fattening in poultry. *J. Nutr.* 127:805S-808S.
- Hermier, D., M. R. Salichon, G. Guy and R. Peresson. 1999. Differential channelling of liver lipids in relation to susceptibility to hepatic steatosis in the goose. *Poult. Sci.* 78:1398-1406.
- Hermier, D., G. Guy, S. Guillaumin, S. Davail, J. M. André and R. Hoo-Paris. 2003. Differential channelling of liver lipids in relation to susceptibility to hepatic steatosis in two species of ducks. *Comp. Biochem. Physiol. Part B.* 135:663-675.
- Hoenig, M., J. B. McGoldrick, M. deBeer, P. N. M. Demacker and D. C. Ferguson. 2006. Activity and tissue-specific expression of lipases and tumor-necrosis factor  $\alpha$  in lean and obese cats. *Domest. Anim. Endocrinol.* 30: 333-344.
- Kadish, A. H., R. L. Little and J. C. Sternberg. 1968. A new rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clin. Chem.* 14:116-131.
- Liu, X. Y., R. G. He, C. S. Huang, X. Li, Q. A. Zhou, C. Wang, N. Zhao and S. X. Zhou. 2006. Hepatic lipogenesis associated with biochemical changes in overfed Landaise geese and china xupu geese. *Agric. Sci. (in China)*. 5(5):390-396.
- Leclercq, B., D. Hermier and M. R. Salichon. 1984. Effects of age and diet on plasma lipid and glucose concentrations in genetically lean or fat chickens. *Reprod. Nutr. Dev.* 24(1):53-61.
- Mourot, J., G. Guy, S. Lagarrigue, P. Peiniau and D. Hermier. 2000. Role of hepatic lipogenesis in the susceptibility to fatty liver in the goose (*Anser anser*). *Comp. Biochem. Physiol. Part B.* 126: 81-87.
- Mulder, M., P. Lombardi, H. Jansen, T. J. Van Berkel, R. R. Frants and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* 268:9369-9375.
- Pedersen, M. E., M. Cohen and M. C. Schotz. 1983. Immunocytochemical localization of the functional fraction of lipoprotein lipase in the perfused heart. *J. Lipid. Res.* 24:512-521.
- Poujardieu, B., R. Rouvier, D. Rousselot-Pailley, G. Guy, A. Rosinski and S. Wezyk. 1994. Croissance et aptitude au gavage de 3 génotypes d'oies. *Ann. Zootech.* 43:197-211.
- Pilo, B. and J. C. George. 1983. Diurnal and seasonal variations in liver glycogen and fat in relation to metabolic status of liver and m. pectoralis in the migratory starling, *Sturnus roseus*, wintering in India. *Comp. Biochem. Physiol. Part A.* 74:601-604.
- Reids, I. M. and C. J. Roberts. 1983. Subclinical fatty liver in dairy cows. *Irish. Vet. J.* 37:104-110.
- Ruffier, L., J. Simon and N. Rideau. 1998. Isolation of functional glucagon islets of Langerhans from the chicken pancreas. *Gen. Comp. Endocrinol.* 12:153-162.
- Saadoun, A. and B. Leclercq. 1987. *In vivo* lipogenesis of genetically lean and fat chickens: effects of nutritional state and dietary treatment. *J. Nutr.* 117:428-435.
- Sato, K., Y. Akiba, S. Kimura and M. Horiguchi. 1995. Species differences between chicks and rats in inhibition of lipoprotein hydrolysis by Triton WR-1339. *Comp. Biochem. Physiol. Part C.* 122:315-319.
- Sato, K., Y. Akiba and M. Horiguchi. 1997. Species difference between chickens and rats in chemical properties of adipose tissue lipoprotein lipase. *Comp. Biochem. Physiol. Part A.* 118: 855-858.
- Sato, K., Y. Akiba, Y. Chida and K. Takahashi. 1999. Lipoprotein hydrolysis and fat accumulation in chicken adipose tissues are reduced by chronic administration of lipoprotein lipase monoclonal antibodies. *Poult. Sci.* 78:1286-1291.

- Whitehead, C. C. and H. Griffin. 1982. Plasma lipoprotein concentration as an indicator of fatness in broilers: effect of age and diet. *Br. Poult. Sci.* 23:299-305.
- Yasuhara, M., T. Ohama, N. Matsuki, H. Saito, J. Shiga, K. Inoue, K. Kurokawa and T. Teramoto. 1991. Induction of fatty liver by fasting in suncus. *J. Lipid. Res.* 32:887-891.