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Effect of Overfeeding on Plasma Parameters and mRNA Expression of Genes Associated with Hepatic Lipogenesis in Geese

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ABSTRACT : The aim of our study was to research the effect of overfeeding on plasma parameters and mRNA expression of genes associated with hepatic lipogenesis in the Sichuan white goose and Landes goose. Fifty-four male Landes geese and 57 male Sichuan white geese were hatched on the same day under the same feeding conditions. After overfeeding for 14 days, (1) extrahepatic adipose tissues grew greatly in the Sichuan white geese, while more lipid accumulated in liver tissue in the Landes geese. (2) Sichuan white geese had a higher plasma concentration of triacylglycerols (TG), lipoproteins and insulin than the Landes geese. However, the Landes geese exhibited higher increase of plasma concentrations of TG, lipoproteins and insulin, with greater decrease of the diacylglycerol acyltransferase 2 (DGAT2) activity and DGAT2 mRNA level and a smller decrease of plasma glucose concentration. In addition, the mRNA level of MTP and LPL in liver was down- and up- regulated by overfeeding, respectively. (3) The correlations between the activity of LPL 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. (4) The proportion of fatty liver weight was positively correlated to plasma concentrations of VLDL and TG in the overfed Sichuan white geese. Such a relationship did not exist in the Landes geese. (5) The activity of DGAT2 and its mRNA abundance in liver had significant negative correlations with the TG content in liver lipid and plasma insulin level in the Landes geese, while in the Sichuan white geese they had negative correlation ($p>0.05$) with TG concentration in liver lipid and had significant positive correlation with VLDL and TG concentrations in plasma. (**Key Words :** Fatty Liver, Goose, Lipids, Overfeeding, Hepatic Lipogenesis)

INTRODUCTION

Under natural conditions, birds, especially some wild waterfowl, are more likely to show non-pathological hepatic steatosis as a result of energy storage before migration (Pilo and George, 1983). This specific capability is used for the production of commercial fatty liver in waterfowl production. Little information is available concerning the mechanism of how fatty liver is induced by overfeeding dietary carbohydrate in palmipedes. Fatty liver induced by overfeeding results from intense lipogenesis which most exclusively occurs in the liver caused by dietary carbohydrate (Davail et al., 2000; Davail et al., 2003a; Molee et al., 2005). When overfeeding-induced lipogenesis intensity exceeds the hepatic capacity for lipoprotein synthesis, a large proportion of the triacylglycerols (TG) will be stored in the hepatocytes and may cause dramatic hepatic steatosis (Fournier et al., 1997; Hermier et al., 2003). During overfeeding, part of the newly synthesized TG are incorporated into hepatic lipoprotein, mainly very low density lipoproteins (VLDL) which can be secreted into blood and used (or stored) in extrahepatic tissues. Therefore, it appears that a liver imbalance between lipogenesis and blood secretion of lipids may mainly explain the hepatic fatty deposition particularly developed in some palmipedes. Interestingly, the susceptibility to fatty liver varies in different species, or even different breeds of palmipedes, even among a given group of birds (Hermier et al., 1991; Poujardieu et al., 1994; Hermier et al., 1999). It was indicated that the breed-related differences of fatty liver susceptibility relies, at least partly, on different genetics in hepatic lipid metabolism and, more specifically, in the channelling of fatty acids towards lipoprotein assembly and secretion. At a molecular level, previous research studied the mRNA expression of malic enzyme (ME) in the lipid synthesis pathway (Mourot et al., 2000; Liu et al., 2006). However, there was no report about the pathway of

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assembly and secretion of lipopretein in the liver of palmipedes, which is another aspect of lipid metabolism.

The present study was designed to elucidate and compare the effects of overfeeding on plasma parameters and mRNA expression of several genes associated with hepatic lipogenesis between Landes goose and Sichuan white goose. Landes goose is famous worldwide for its fatty liver production, and Sichuan white goose has a moderate capability for egg laying and meat production in China. Several plasma parameters (lipoproteins, TG, glucose and insulin) were measured to study their influence on hepatic lipid synthesis and secretion. Meanwhile, the related enzyme activity and gene expression of lipoprotein lipase (LPL), diacylglycerol acyltransferase 2 (DGAT2) and microsomal triglyceride transfer protein (MTP) were studied regarding their regulation of VLDL-TG assembly and secretion. This would be a strong asset to breeding of specialized strains of geese for fatty liver and production of the commercial foiegras.

MATERIALS AND METHODS

Animals and experimental design

Fifty-four male Landes geese and 57 male Sichuan white geese hatched on the same day were grown under natural conditions of light and temperature at the Experimental Farm for Waterfowl Breeding of Sichuan Agricultural University. The two breeds were housed collectively in separated pens. In order to get a more valid comparative study of fat storage and metabolism between the two breeds, the amount of food provided to the animals was related to their body weight (on average 25 g/day per 100 g of BW). From 0 to 4 week of age, the geese were fed *ad libitum* a starting diet, containing 12 MJ/kg and 20.5% protein, and from 4 to 14 weeks a growing diet, containing 11 MJ/kg and 13.8% protein, with a progressively reduced daily intake to avoid excessive fatness. At the end of the pre-overfeeding week, each breed was separated into control and overfed groups. The control group continued to have a free-growing diet, and the overfed group were given four meals a day for 14 days of a carbohydrate-rich diet consisting of boiled and salted maize (14 MJ/kg, 9% protein, and 0.45% fat) with 0.4% waterfowl fat and water added. Birds had free access to water at any time. Sichuan white geese, having the lower capacity for overfeeding ingestion, were fed by the operator at the maximum of their ingestion potential. The mean value of their daily food intake (as g of food/kg BW) was used to calculate the food intake of the Landes geese as described by Davail and his colleagues (2003b). During the overfeeding period, geese were housed in individual cages having free access to water at all times. In the overfeeding room, temperature was 15-18°C and hygrometry was 70-80%.

On the last day of overfeeding (16 weeks), geese were provided with water but deprived of feed overnight for 18 h and provided with water. On the following morning, blood was withdrawn by puncture of the occipital venous sinus, collected on EDTA (1.2 g/L) in a vacuum tube and kept at 2 to 4°C during the subsequent procedures. Individual plasma samples were separated by centrifugation at 2,000×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 was frozen at -20°C until further analyses.

Immediately after blood sampling, the geese were killed by exsanguination. A sample (20 g) was immediately taken from the ventromedial portion of the main lobe (right lobe) of each liver, through a limited incision of the abdominal wall. The samples were frozen in liquid nitrogen and stored at -80°C until analysis of enzymatic activity and mRNA level. The corresponding carcasses were then kept at 4°C overnight before dissecting and weighing representative body compartments: the liver, abdominal adipose tissue and associated subcutaneous adipose tissue (skin included).

Liver 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 with petroleum ether at 40 to 65°C. After weighing, liver lipid was extracted and preserved in the solution of chloroform-methanol (9:1, v/v) from another freeze-drying of 1 to 2 g of liver and stored at -20°C. TG content in liver was assayed by the acetylacetone method (Chen, 1999).

Plasma analyses

Triglycerides (TG), glucose, insulin and activity of lipoprotein lipase in whole plasma were determined using corresponding kit. All kits were provided by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. Lipoproteins were separated from plasma by ultracentrifugation for 18 h at 10°C and 175,000 g and the concentration was determined as described previously (Fournier et al., 1997).

Measurement of DGAT2 activity

DGAT2 activity was assayed with 3.67 μM ^{14}C-18:1-Coenzyme A and 1.5 mM 1,2-18:1 diacylglycerol (DAG) (prepared as a 150 mM stock in 2-methoxyethanol) in a buffer containing 10 mM potassium phosphate (pH 7.0), 150 mM KCl, and 0.1%TX-100 (w/v) in a total volume of 100 μl as described by Kamisaka et al. (1993) and Kamisaka and Nakahara (1994). Assays were performed at 30°C for 5 min and terminated with the addition of 1.5 ml of heptane:isopropanol:0.5 M H_2SO_4 (10:40:1, v/v/v). After

Table 1. Influence of overfeeding on body composition

	Control group		Overfed group	
	Landes (n = 32)	Sichuan (n = 30)	Landes (n = 18)	Sichuan (n = 26)
Birth weight (g)	100.4±9.15***	90.96±5.46	103.52±11.73***	91.64±6.02
Body weight at 16 weeks (g)	4,815.14±368.6***	4,133.33±318.49	6,051.33±514.75***,^{b}	5,111.3±420.52^{c}
Liver weight (g)	90.57±16.08	83.00±11.86	519.33±124.31***,^{c}	310.74±79.94^{c}
Liver weight (% BW)	1.89±0.31	2.01±0.26	8.6±2.1*,^{c}	6.1±1.6^{c}
Lipid proportion (% LW)	3.26±1.73*	1.88±0.58	49.06±7.31**,^{c}	38.47±7.16^{c}
TG content of lipid (%)	8.21±0.90**	5.32±0.47	94.07±0.99**,^{c}	90.92±2.34^{c}
Scat+skin (g)	812.86±26.98	607.00±27.25	1,273.3±221.1*,^{c}	1,110.4±189.0^{c}
Scat+skin (% BW)	16.85±2.03	14.62±1.70	21.0±3.8^{a}	21.6±3.7^{a}
Abdominal fat pad (g)	145.00±7.01	80.80±9.57	265.5±59.0^{c}	234.8±70.5^{c}
Abdominal fat pad (% BW)	3.01±0.49	1.94±0.62	4.4±1.0^{a}	4.5±1.4^{b}

BW = Body weight, LW = Liver weight, Scat = Subcutaneous adipose tissue.

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

$^{a, b, c}$ Effect of overfeeding at $p<0.05$, $p<0.01$ and 0.001, respectively.

the assays were terminated, the samples could be stored at 4°C for processing at a later date or immediately processed by addition of 0.1 ml 1 M $NaHCO_3$ followed by 1 ml of heptane containing 15 nmol/ml triolein as a carrier for extraction. The samples were vortexed and, after separation of aqueous and organic phases, the upper organic phase was removed into a new glass vial and washed with 1 ml 1 M NaCl. Forty percent of the final organic phase was removed for liquid scintillation counting and the remaining organic phase was transferred into a clean vial and evaporated to dryness under nitrogen gas. The residue was resuspended in 45 μl hexane and spotted onto a silica gel-G glass thin-layer chromatography (TLC) plate with a pre-adsorbent loading zone. The TLC plate was developed in hexane:diethyl ether:acetic acid (50:50:1, v/v/v) to the top then dried and scanned by a formatter to determine the portion of radioactivity incorporated into triacylglycerol.

Gene expression

Gene expression was determined by semi-quantitative RT-PCR. Total RNA was isolated from individual livers using Trizol reagent (Invitrigen) according to the manufacturer's instruction. Semi-quantitative RT-PCR was performed to determine the presence and relative level of DGAT2, LPL and MTP mRNA using constitutive expressed gene, 18 s, as a positive control.

Statistical analysis

Results were expressed as means±SD. The data were subjected to ANOVA (SAS, 1999) and the means were compared for significance by Tukey's test. Correlations were determined by linear regression (SAS, 1999).

RESULTS

Body composition

As shown in Table 1, the body weight of 16 week-old geese in the overfed group was significantly higher ($p<0.001$) than that in control group, indicating that overfeeding had a remarkable effect on the body weight of the two breeds. The increased proportion of abdominal adipose tissue and subcutaneous adipose tissue (including skin) was even more evident in the Sichuan white geese compared to Landes geese. The subcutaneous adipose tissue (including skin) weight of the Landes geese was higher ($p<0.05$) than the Sichuan white geese in the overfed group, while there was no significant difference between the control groups.

Liver analyses

As shown in Table 1, liver weight of the Landes geese and the Sichuan white geese in the overfed group was 5.73 times and 3.74 times, respectively, that in the control group and was significant higher in the Landes geese than in the Sichuan white geese. Liver proportion was 4.5 times and 3 times that of the control group, respectively, in Landes and Sichuan white geese. Lipid proportion of the Landes geese and Sichuan white geese had increased 13.67 times and 19.46 times, respectively, and TG content of lipid also significantly increased. The lipid proportion in Landes goose was significantly higher than in Sichuan white goose, as was TG content in lipid. These observations indicate that overfeeding can stimulate TG to accumulate in liver and induce the liver steatosis to form fatty liver.

Plasma parameters

Plasma concentrations of lipoproteins and TG were significantly higher than in the control group (Table 2). In both control and overfed groups, plasma concentrations of VLDL and TG in Sichuan white geese were markedly higher than in Landes geese.

Glucose concentration in plasma was significantly higher in Sichuan white geese than in Landes geese in the control group. In the overfed group, the Sichuan white

Table 2. Effect of overfeeding and strain of goose on blood chemicals (Unit: mmol/L)

	Control group		Overfed group	
	Landes (n = 35)	Sichuan (n = 30)	Landes (n = 19)	Sichuan (n = 27)
VLDL	0.70±0.11	1.11±0.10**	1.70±0.19[a]	2.20±0.17[a, **]
LDL	0.86±0.15*	1.37±0.30	2.25±0.45[*, b]	2.77±0.53[b]
HDL	0.23±0.02	0.54±0.05	3.11±0.51[*, b]	3.68±0.56[b]
TG	1.53±0.23	2.43±0.22**	3.71±0.43[a]	4.87±0.36[b, *]
Glucose	5.14±0.98	7.53±0.49**	6.35±1.52[***, a]	2.76±1.31[b]
Insulin	6.61±0.23	6.61±0.25	10.28±0.31[b]	11.53±0.28[*, b]

*, ** Difference between Landes and Sichuan white geese at $p<0.05$ and 0.01 respectively.
[a, b, c] Effect of overfeeding at $p<0.05$, 0.01and 0.001, respectively.

Table 3. Enzymes activities and mRNA abundance in liver

	Control group		Overfed group	
	Landes	Sichuan	Landes	Sichuan
DGAT2 activity (A) (n = 16)	3.49±0.84[*, b]	3.19±0.54[a]	2.24±0.45	2.95±0.41*
Activity of plasma LPL (B) (n = 16)	4.98±0.72	4.75±0.63	7.61±2.13[b]	11.40±3.06[***, b]
DGAT2 mRNA level (n = 3)	1.66±0.11[**, a]	1.48±0.27[a]	1.23±0.11	1.41±0.10*
MTP mRNA level (n = 3)	1.13±0.10[a]	1.00±0.12[a]	1.05±0.11	0.975±0.14
LPL mRNA level (n = 3)	0.98±0.14	0.98±0.13	1.50±0.08[b]	1.34±0.11[a]

A: 10^{-1} nmol Tripalmitoylglycerol/min/mg microsomal protein. B: μmol palmate/ml·h.
*, ** Difference between Landes and Sichuan white geese at $p<0.05$ and 0.01 respectively.
[a, b] Effect of overfeeding at $p<0.05$and 0.01, respectively.

Table 4. The correlation between body composition and plasma VLDL, TG concentrations and Lipoprotein lipase activity after overfeeding

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

The numbers in the table express correlation coefficients. * $p<0.05$; ** $p<0.01$.
BW = Body weight, 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.

geese had markedly higher insulin concentration but lower glucose concentration in plasma, compared to Landes geese (Table 2).

Activities of lipogenic enzymes and the level of mRNA expression

The enzyme activities and mRNA expression are shown in Table 3. Overfeeding resulted in a remarkable increase of LPL activity in the two breeds. 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. The LPL mRNA level of the overfed group in both breeds was higher ($p<0.05$) than in the control group. There was no difference between the two breeds in either the control or overfeeding groups.

Compared to the control group, overfeeding significantly decreased ($p<0.05$) DGAT2 enzyme activity and DGAT2 mRNA level in the two breeds. Landes geese had higher DGAT2 activity in the control group, while they had lower DGAT2 activity in the overfed group (Table 3).

In the two breeds, MTP mRNA level in the overfed group was lower ($p<0.05$) than in the control group. MTP mRNA level of Landes geese was higher ($p<0.05$) than in Sichuan white geese in both control and overfed groups (Table 3).

Correlation analysis

In Sichuan white geese, the proportion of fatty liver weight was positively correlated with plasma TG and VLDL concentrations (Table 4). These correlations were not significant in Landes geese. In the two breeds, the concentration of TG was positively correlated with plasma VLDL concentration. In the overfed group, the activity of LPL in Sichuan white geese was positively correlated with the proportions of subcutaneous adipose tissue and the

Table 5. The correlation between enzyme activities, mRNA abundance of DGAT2 and several parameters after overfeeding

	DGAT2 activity		DGAT2 mRNA level	
	Landes	Sichuan	Landes	Sichuan
Insulin concentration in plasma	-0.52*	-0.37	-0.53*	-0.40
Glucose concentration in plasma	-0.22	0.35	-0.24	0.39
VLDL concentration in plasma	0.48	0.75**	0.43	0.52*
TG concentration in plasma	0.48	0.81**	0.42	0.57*
TG content in liver lipid	-0.63*	-0.38	-0.68*	-0.30

The numbers in the table express correlation coefficients.
* $p<0.05$; ** $p<0.01$.

proportions of abdominal adipose tissue and was negatively correlated with the plasma concentration of VLDL, 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 liver weight and was positively correlated with the proportions of subcutaneous adipose tissue, but had no evident correlation with the proportions of abdominal adipose tissue and the plasma concentration of VLDL.

As shown in Table 5, plasma insulin concentration of Landes geese had a negative correlation with the expression of DGAT2 mRNA and its enzyme activity. In the overfed group, the activity of DGAT2 and its mRNA abundance in liver had a significant negative correlation with TG content in liver lipid, but had no marked correlation with VLDL and TG concentration in plasma in Landes geese. However, in Sichuan white geese the activity of DGAT2 enzyme and its mRNA abundance in liver not only had a negative correlation with TG content in liver lipid, but had a significant positive correlation with the VLDL and TG concentration in plasma.

DISCUSSION

Our results showed that overfeeding resulted in stronger development of extrahepatic adipose tissues in the Sichuan white goose, while there was more lipid accumulation in liver tissue of the Landes goose. Both liver and adipose tissue in birds are sites of fatty acid synthesis, but the liver is the main site (Saadoun and Leclercq, 1987), which may be one reason for the more sensitive effect of overfeeding on liver than on adipose tissue in the goose. DGAT2, MTP, LPL have an important role in the assembly and secretion of VLDL-TG in liver. Overfeeding induced a decreased enzyme activity of DGAT2 and decreased mRNA expression of DGAT2 and MTP, and an increased LPL mRNA level in liver in the two breeds, which could suppress the assembly and secretion of VLDL-TG in liver and degree of stearolysis of VLDL-TG in plasma. This in turn hampered the secretion of VLDL-TG in liver, and the impaired utilization of plasma VLDL-TG caused more compensatory transfer and storage of TG in the liver, so TG content in liver was suddenly raised and then a dramatic hepatic steatosis occurred. Only the mRNA expression of DGAT2 in the three genes was different between the two breeds. Meanwhile, the mRNA expression and enzyme activity of DGAT2 in the overfed Landes geese had a negative correlation with plasma insulin level, which indicated that the increased insulin caused down regulation of mRNA expression and enzyme activity of DGAT2, and suppressed the normal secretion of VLDL-TG. In addition, mRNA expression and enzyme activity of DGAT2 in the overfed Landes geese had a negative correlation with the TG content in liver lipids, but in Sichuan white geese it had a striking correlation with the plasma VLDL-TG concentration. These findings suggest that mRNA expression and enzyme activity of DGAT2 mainly affected the TG content of liver lipids in Landes geese, but influenced the plasma VLDL-TG concentration in Sichuan white geese. Therefore, the difference in DGAT2 gene expression between the Landes and Sichuan white geese may explain, at least partially, the difference in susceptibility to fatty liver and fatty deposition in adipose tissue.

The Sichuan white geese had a higher concentration of lipoproteins which suggests that this breed reacts to overfeeding by enhancing export of all liver lipids. The greater amount of subcutaneous and abdominal tissues in this breed supported this hypothesis, because the development of adipose tissue in birds depends mostly on the uptake of VLDL which was synthesized and secreted by the liver (Hermier et al., 1991). Overfeeding enhanced lipoprotein secretion in Sichuan white geese, which limited hepetic steatosis and promoted extrahepatic fatty deposition. In Landes geese a failure in the channelling of hepatic lipids towards secretion in plasma and peripheral adipose storage was favourable to the establishment of steatosis. In addition, in overfed Sichuan white geese, plasma TG and VLDL concentrations were positively correlated with the proportion of fatty liver weight. As in other avian species (Hermier, 1997), these positive correlations indicate that plasma VLDL concentration is a good indicator of hepatic lipogenesis and suggest a relatively good balance between hepatic lipogenesis and lipoprotein export to peripheral tissues, which may be a limiting factor in the susceptibility to fatty liver in Sichuan white geese. In contrast, the

absence of a similar correlation in Landes geese was consistent with the preferential storage of lipids in liver, probably because the incorporation of newly synthesized TG into lipoproteins is partly impaired in Landes geese (Hermier et al., 1991).

The activity of LPL is critical to the growth and the development of adipose tissue. The overfed Sichuan white geese displayed strikingly higher plasma concentrations of VLDL, TG and activity of LPL. These data indicate that in the adipose tissue of Sichuan white geese, the intensity of reaction to hydrolyze VLDL-TG into free fatty acids catalyzed by LPL was much stronger, which could explain why overfed Sichuan white geese have a powerful capability of adipose tissue development. In contrast, the lower activity of LPL in overfed Landes geese may be a limiting factor to extrahepatic fattening in this breed. TG, which was not catabolized by LPL, may be returned to the liver induced by some lipoprotein where it is assimilated to promote hepatic steatosis mediated by a specific lipoprotein receptor.

In summary, overfeeding induced in Landes geese a huge capability for hepatic steatosis, while Sichuan white geese had a stronger development of adipose tissue. The different LPL activity, plasma VLDL concentration and the difference of DGAT2 activity and gene expression between Landes and Sichuan white geese may be the reasons for their different susceptibility to fatty liver. The regulation of the MTP, DGAT2 and LPL gene expression induced by overfeeding in liver may be associated with hepatic steatosis.

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