Analysis of mRNA expression of genes related to synthesis of fatty acids in goose fatty liver

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Abstract

The aim of our study was to evaluate the effect of overfeeding on mRNA expression levels of genes involved in lipogenesis, in order to understand the mechanism of hepatic steatosis in the goose. Using Landes goose (Anser anser) and Sichuan White goose (Anser cygnoides) as experimental animals, we quantified the mRNA expression of lipogenic genes, acetyl-CoA carboxylase-α (ACCo) and fatty acid synthase (FAS), and of two transcription factors, sterol regulatory element-binding proteins-1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP) by real-time polymerase chain reaction (RT-PCR), and measured the lipid and triglyceride (TG) content in the liver and also of the plasma level of glucose, insulin and TG. Our results indicated that compared to the control group, the overfeeding induced an increase of the lipid and TG content in the liver and also of the plasma insulin and TG concentration in both breeds. However, the plasma glucose level decreased after overfeeding in the Sichuan White goose, and there was no evident change in the Landes goose. Lastly, the mRNA expression of ACCα, FAS, SREBP-1 and ChREBP in the overfed group was lower than in the control group in both breeds. We concluded that the lipogenesis pathway plays a role in overfeeding-induced hepatic steatosis and that the decreased mRNA level of related genes may be the indicator of hepatic steatosis.

Introduction

In some waterfowl species, overfeeding produces an intense lipogenesis that occurs in the liver almost exclusively. Much of the synthesised triacylglycerols is stored in the hepatocytes and may cause a dramatic hepatic steatosis. The mechanisms underlying hepatic steatosis include an oversupply of free fatty acids to the liver, interference with the triglyceride cycle, increase in the synthesis or esterification of fatty acids, decreased fatty acid oxidation, decreased apoprotein synthesis, and decreased synthesis or secretion of very low density lipoprotein (Treinen-Moslen, 2001). Excessive accumulation of triglycerides (TG) is the main characteristic of hepatic steatosis, a pathological pattern that is now considered as a component of the metabolic syndrome (Marchesini et al., 2001). Fatty acids utilised for TG synthesis in the liver are available from the plasma non-esterified fatty acid pool (NEFA) but also from newly synthesised fatty acids (Dentin et al., 2005). Thus, the enhanced hepatic fatty acid synthesis is an important metabolic pathway leading to the development of hepatic steatosis (Pistis and Girard, 2008b). Many, if not all, of these effects are a result of alterations in gene expression, which can be detected easily in the laboratory and used as biomarkers for breeding.

A key role in the hepatic fatty acid synthesis and steatosis is played by the transcription factors sterol regulatory element-binding proteins-1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP) by real-time polymerase chain reaction (RT-PCR), and measured the lipid and triglyceride (TG) content in the liver and also of the plasma level of glucose, insulin and TG. Our results indicated that compared to the control group, the overfeeding induced an increase of the lipid and TG content in the liver and also of the plasma insulin and TG concentration in both breeds. However, the plasma glucose level decreased after overfeeding in the Sichuan White goose, and there was no evident change in the Landes goose. Lastly, the mRNA expression of ACCα, FAS, SREBP-1 and ChREBP in the overfed group was lower than in the control group in both breeds. We concluded that the lipogenesis pathway plays a role in overfeeding-induced hepatic steatosis and that the decreased mRNA level of related genes may be the indicator of hepatic steatosis.

Materials and methods

Animal and experimental design

The Landes goose is a European breed and famous for a high capability of fatty liver production, and the Sichuan White goose is a Chinese breed with a good capability for egg laying and meat production. Fifty-seven male Sichuan White goose (Anser cygnoides) and 54 male Landes goose (Anser anser) hatched on the same day, and the two breeds were housed collectively in individual rooms. From 0 to 4 weeks of age, the geese had free access to a starting diet containing 2900 kCal/kg and 20.5% protein, and from 4 to 14 weeks to a growing diet containing 2600 kCal/kg and 13.8% protein. The daily intake was progressively reduced to prevent them from becoming excessively fat. The diet of the Sichuan White goose was reduced by 200 g/day and that of the Landes goose by 250 g/day between 4 and 5 weeks; Sichuan White goose 300 g/day and Landes goose 375 g/day between 5 and 8 weeks; and Sichuan White goose 150 g/day and.
Landes geese 200 g/day between 8 and 13 weeks. At 14 weeks of age, they began the period of ‘preoverfeeding’ for one week, which allowed the time amount of food fed progressively increased (an average of 400 g/day for Sichuan White geese and 500 g/day for Landes geese) to increase the volume of the digestive tract and to initiate the metabolic adaptation to overfeeding. At the end of the preoverfeeding week, both breeds were divided into two groups, the control group and the overfed group. The control group continued to have a free growing diet, and the overfed group was given four meals a day of a carbohydrate-rich diet consisting of boiled and salted maize (3370 kCal/kg, 9% protein and 0.45% fat) with 0.4% waterfowl fat and water added for 14 days. The geese had free access to water. The Sichuan White geese, having the lower capacity for overfeeding ingestion, were fed the maximum of their ingestion potential by the operator. The mean value of their daily food intake (g of food/kg BW) was used to calculate the food intake of the Landes geese, as described by Davail et al. (2003). In order to ensure a more valid comparison of the metabolism between the two breeds, the amount of food provided to the geese was related to their body weights (on average 25 g/day per 100 g of BW) in the experiment (Sichuan White geese were given 1000 g/day and Landes geese 1100 g/day, on average). During the overfeeding period, the geese were housed in individual cages and had free access to water. In the overfeeding room, the temperature was 15-18°C and humidity 70%-80%.

On the last day of overfeeding (at 16 weeks), the geese were deprived of feed overnight. On the following morning, blood was withdrawn and the plasma was frozen at -20°C for further analysis. Immediately after blood was sampled, the plasma was frozen at -20ºC for further analysis. Immediately after blood was sampled, the plasma was frozen at -20ºC for further analysis.

Measurement of total lipid and triglyceride content

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

Measurement of plasma parameters

Plasma glucose was measured enzymatically using a Beckman autoanalyzer (Kadish et al., 1968) and plasma insulin levels were determined by a radioimmunoassay with a guinea pig-porcine insulin antibody, using chicken insulin as the standard (Ruffier et al., 1998). Plasma TG concentration was quantified in whole plasma by colourimetric enzymatic methods (Fossati and Prencipe, 1982), and all the kits were provided by the Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. Analyses were performed in duplicates.

Results

Effect of overfeeding on lipid deposition in the liver

Because the body weights and liver weights of the two breeds were very different between the control group and the overfed group, it was more informative to express the liver weight as a percentage of the body weight. As shown in Figure 1, the liver weight proportion of both breeds in the overfed group was much higher than that in the control group, as expected. Overfeeding induced an evident increase of the lipid proportion in the liver and of the TG content of the livers. Moreover, the increase of the liver weight and lipid proportion in the liver by overfeeding was more evident in the Landes goose than in the Sichuan White goose, indicating that the Landes goose had...
much greater liver steatosis than the Sichuan White goose.

**Effect of overfeeding on plasma triglyceride, glucose and insulin concentration**

As shown in Figure 2, overfeeding had an evident effect on the concentration of plasma TG, glucose and insulin, and there was a difference in the effect between the Landes goose and the Sichuan White goose. The inducting effect on insulin was greater in the Sichuan White goose than in the Landes goose (P<0.05). Interestingly, overfeeding had no evident effect on the glucose level in the Landes goose, but was evidently decreased in the Sichuan White goose. There was no apparent difference in the effect of overfeeding on the plasma TG concentration between the two breeds.

**Discussion**

Lipogenesis is the metabolic pathway leading to the conversion of an excess of carbohydrates into fatty acids, which are ultimately esterified with glycerol-3-phosphate to form TG. The activity of the lipogenic pathway is strongly dependent on nutritional conditions, and it has been clearly established that lipogenic enzyme transcription requires both insulin and glucose to be fully induced (Foufelle and Ferré, 2002). Conditions associated with high rates of lipogenesis such as a low-fat/high-carbohydrate (LF/HC) diet are associated with a shift in cellular metabolism from lipid oxidation to TG esterification, thereby increasing the availability of liver TG. Overfeeding the goose could induce the imbalance of insulin and glucose metabolism, which may have a close relationship with the increased lipid deposition in the goose liver.

The use of transgenic, knockout and knockdown mouse models has helped, over the years, to achieve a better understanding of the molecular determinants of hepatic steatosis (Postic and Girard, 2008a). Key enzymes of fatty acid synthesis such as ACCα and SCD1 have been shown, when knocked down, to reverse many of the metabolic defects associated with hepatic steatosis (Gutierrez-Juarez et al., 2006; Savage et al., 2006), indicating that the expression of lipogenic genes in the liver is important for the information of hepatic steatosis. Lipogenic gene expression is coordinately controlled by key transcriptional regulators: SREBP-1 and ChREBP (Dentin et al., 2005; Foufelle and Ferré, 2002). Induction of lipogenic genes (ACCα and FAS) is under the concerted action of ChREBP and SREBP-1c in response to nutritional signals (Foufelle and Ferré, 2002; Postic and Girard, 2008b).

In our study, after overfeeding for 14 days, the mRNA expression of lipogenic genes (ACCα and FAS) and two transcription factors (SREBP-1 and ChREBP) was decreased. It is supposed that in the process of overfeeding, the level of those genes increases. The decrease in SREBP-1c and ChREBP suggests that this pair of transcription factors is involved in the inhibitory effect of overfeeding on lipogenic genes. After overfeeding for 14 days, the amount of TG accumulated quickly in the liver, and the metabolic capability of the goose hepatocytes was impaired; this may induce the decrease in lipid deposition. In general, excess carbohydrate could induce an increase of de novo lipogenesis, and a four-day carbohydrate overfeeding in humans increased the expression of mRNAs coding for SREBP-1c, FAS and

**Table 2. mRNA abundance in goose liver.**

| Control group | Overfed group |
|---------------|---------------|
| Landes, n=3   | Sichuan, n=3  | Landes, n=3 | Sichuan, n=3 |
| ACCα          | 1.519±0.189A  | 1.494±0.207A | 1.000±0.098B  | 0.915±0.165A  |
| FAS           | 1.240±0.127B  | 2.759±0.325A | 1.000±0.110B  | 2.098±0.219B  |
| SREBP-1       | 2.487±0.261A  | 1.769±0.203B | 1.000±0.100B  | 1.085±0.107A  |
| ChREBP        | 2.911±0.257A  | 1.372±0.155B | 1.000±0.099B  | 0.961±0.132A  |

A,B,a,b Different letters indicate the difference at P<0.05.
Conclusions

In conclusion, overfeeding the goose could induce the imbalance of insulin and glucose metabolism, which may have a close relationship with the increased lipid deposition in the goose liver. The Landes goose and Sichuan white goose have different responses to overfeeding, which may be a result of their different capabilities of lipid deposition.

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ACC (Minehira et al., 2003). Although de novo lipogenesis may be stimulated through an increase in lipogenic enzyme activity even in the absence of changes in lipogenic enzymes on RNA levels, the marked increase in TG content in the goose liver after overfeeding indicated that carbohydrate overfeeding also increased lipogenic enzyme transcription. The decreased level of lipogenic genes and related transcription factors after overfeeding of 14 days indicated the lipid deposition began to decrease after the rapid increase of lipogenesis, which may be the indicator of hepatic steatosis, but this needs further verification.

The liver is the central organ in the disturbances in glucose and lipid metabolism. Although many studies have shown strong associations between hepatic TG content and hepatic insulin resistance (Marchesini et al., 2005; Seppala-Lindroos et al., 2002), few studies have investigated the mechanisms underlying this association. The fatty liver is considered as a mediator in the perturbations of glucose and lipid metabolism. Hepatic steatosis can be both actively and passively involved in these metabolic disturbances. The glucose level in the overfed Sichuan White goose is lower than in the overfed Landes goose, indicating the intensity of TG synthesis by the phosphatidylglycerol pathway is lower in the Sichuan white goose, followed by a lower lipid deposition in liver. Meanwhile, the increase in the plasma insulin level induced by overfeeding is greater in the Sichuan White goose than in the Landes goose. This interesting phenomenon indicates that the two breeds have a different response to overfeeding, which may be a result of their different capabilities of lipid deposition. We propose that the lower plasma glucose level in the Sichuan White goose may promote the secretion of pancreatic glucagon, thereby resisting the role of insulin, which limits the transformation of glycogen to fatty acids and the deposition of lipids in the liver. This possibility may be the reason that the Sichuan White goose has a lower degree of hepatic steatosis.