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RT-qPCR Expression of Fatty Acid Binding Protein1 and 2 (FABP1&2) Genes in Fatty Liver Tissue Overfed Goose

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Abstract

Fatty acid-binding proteins 1, 2, 3, 4 and L-FABP occur in the liver in a high concentration where it is involved in the fatty liver functions. In this study, diet content cooked maize was used to overfeed Landes geese for 19 days. The expression levels of *FABP1/2* were determined by using quantitative PCR in goose liver and primary hepatocytes. The expression level of *FABP2* gradually decreased with overfeeding time in the livers of the geese, while *FABP1* was extremely down-regulated after one week of overfed. The expression level of *FABPs1* mRNA was the lowest in the overfeeding group at 89 days. In primary hepatocytes, the data indicated that the expression of *FABP1/2* were inhibited by high levels of glucose (25-50 mM). In addition, insulin (50-100mM) inhibited *FABP1* in goose hepatocytes, while it was not significantly (p 0.05) for *FABP2*. These findings suggest that the expression of the *FABP1* gene is hyper-effect to hyperglycemia and hyperinsulinemia that causing inhibition of the gene in goose fatty liver. For fatty acid treatment, the expression levels of *FABP1/2* were not significantly altered in fatty acid (0.25 or 0.5mM) treatment. All data were normalized by the *GAPDH* gene and analyzed by the $2^{-\Delta\Delta Ct}$ method.

Keywords

Fatty Liver, Goose, *FABP 1/2 genes*, Overfeeding, Primary Hepatocytes

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1. Introduction

The small cytosolic proteins that bind in long-chain fatty acids with sub-micromolar affinities are called fatty acid-binding protein (*FABP*) [1, 2], including a large multigene family of highly abundant, cytosolic lipid-binding proteins which play an important role in the coordination of the intracellular lipid trafficking, as well as regulate the metabolic and inflammatory pathways [3, 4]. the dominant *FABP* in mammalian intestine, liver fatty acid-binding protein (*L-ABP*) plays a critical role in intestinal fatty acid trafficking and compartmentalization [5]. Beyond a role in modulating lipid flux, while, the expression of *L-FABP* within colorectal cancer tumor tissue has been linked to histologic (tumor) differentiation, the incidence of lymph node metastasis, and overall prognosis [6, 7]. The previous

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studies imply that *FABP* expression may modulate signaling pathways involved in colorectal cancer initiation and progression, although the mechanisms are currently unknown. However, among the *FABPs*, *L-FABP* is highly distributed in mammal *FABP* and it is mostly expressed in the tissues and active when unesterified long-chain fatty acid (LCFA) metabolism is low [8]. The biological function and metabolic mechanism of the L-FABP gene have been well-studied in mammals. However, there is a paucity of information regarding the function and mechanism of L-FABP in goose, it may the unusual fatty acid metabolism system in these birds. in mammals, the structure and functions of the L-FABP is more efficient than the other types of the FABPs, while in goose each L-FABP molecule binds 2 fatty acids, whereas other FABPs can only bind one fatty acid.

Landes geese are considered good fatty liver production, and the average of its liver weight after overfeeding is approximately between 600 to 800 g in a short time. According to the previous studies, FAS plays an important role in fatty acid synthesis [9], and its activity is regulated by insulin signaling, fatty acid and glucose [10].

In this research, a high carbohydrate diet was used to overfeed Landes geese for 19 days. The livers were used for transcriptome analysis with RNA-sequencing (RNA-seq) technology. The goose primary hepatocytes were treated with fatty liver-related factors which containinghigh levels of glucose, insulin and fatty acids. The results were confirmed by quantitative PCR. To understand how the fatty acid-binding proteins genes were up-regulated in fatty liver context, together, the present study suggests that the reduction of fatty acid-binding proteins genes expression is required for the development of goose fatty liver.

2. Materials and Methods

2.1. Animals Experiment

One-day-old healthy Landes geese were purchased from Wu Wang Farm (Chuzhou, China) and were randomly divided into two groups, i.e., a control and an overfeeding group. The geese were raised under the condition of natural light and temperature. The control geese were allowed *ad libitum* feeding of cooked maize, while the overfed geese were provided with a diet that contains cooked corn, 1% plant oil and 1% salt. The formal overfeeding was beginning at 70-day-old. of and the following protocol was applied: in the first 5 d the daily feed intake (3 meals a day) reached 500 g, followed by 800 g of daily feed (4 meals a day) for the following week and 1,200 g of daily feed intake (5 meals a day) for the remaining days. The geese were slaughtered at the age of 70, 77, 84 and 89 days. The livers from the geese were snap-frozen in liquid nitrogen and stored at -70°C until use. All animal protocols were approved by the Yangzhou University Animal Ethics Committee.

2.2. Preparation of Goose Primary Hepatocytes

Hepatocytes were isolated from Landes goose embryos at 23 days. The protocol for hepatocyte isolation is briefly described [11]. The cells were diluted with culture medium to 1×10^6 cells/ml, plate 1×10^6 cells per well in 12-well dishes, followed by incubation in 5% CO_2 at 38°C until treatment. The media was renewed at first 6 h of incubation and every 24 h for later incubation.

2.3. Treatment of Cultured Goose Primary Hepatocytes with Glucose, Fatty Acids, and Insulin

Primary hepatocytes were isolated from embryo's liver and then cultured for 24 h before any treatments. The treatments of the culture performed according to the method described by [11]. Control cells were treated by serum-free culture media containing 11.61L/mL of HCl. All the primary hepatocytes were rinsed with phosphate buffered saline (PBS) twice and then the cells were harvestied at the end of treatment with 1 mL TRIzol Reagent (Cat. No. 15596026, Life, USA) per well.

2.4. Isolation of Total RNA and Amplification to cDNA

Total RNA was extracted by TRIzol (TIANGEN BIOTECH (Beijing) CO., LTD) from the liver of the overfeeding and control group, according to the manufacturer's instructions. RNA was eluted in nuclease-free water and then subjected to DNAase treatment to remove genomic DNA. Both the quality and quantity of total RNA were assessed at OD A260/A280 values and then were ranged between 1.8 to 2, indicating a high-quality RNA using a NanoDrop® spectrophotometer (Nano Drop Technologies, Inc. Wilmington, DE, USA). Samples were stored at −80 ºC before reverse transcription was performed. RNA was reverse-transcribed to 2μg per sample according to the manufacturer's instructions SuperQuickRT cDNA kit (CWBIO). The cDNA was stored at −20°C until analysis.

2.5. Expression of mRNA Abundance by Quantitative PCR

The genes expression were previously described [12]. Briefly, expression levels of *FABP1/2* genes in the liver of the overfed and normally fed geese, as well as the treated goose primary hepatocytes was performed with SYBR® Green Master Mix kit (Vazyme Biotech Co., Ltd). The primer for quantitative PCR was listed in Table 1. The glyceraldehyde-3-phosphate dehydrogenase gene (*Gapdh*) gene was used as

an internal control gene for normalization. The cycle threshold (Ct) was determined with the supplied software. The relative mRNA abundance of genes of interest was calculated using $2^{-\Delta\Delta Ct}$ and presented as fold change over control using the method previously described [13].

Table 1. Primers used in this study.

Gene	Forward 5'-3'	Reverse 5'-3'	Product size
FABP1	AAAACACCCAAGCAATCT	TCTGACAAGCGTTACTCC	207 bp
FABP2	TGGAGCAACGCTGAAGAG	TTGACAAGGCTGGAGACC	104 bp
GAPDH	GCCATCAATGATCCCTTCAT	CTGGGGTCACGCTCCTG	200 bp

2.6. Bioinformatics Analysis

fatty acid binding protein 1/2 genes primers were designed by Premier5® software (PremierBiosoft, Palo Alto, CA, USA) and figures were constructed by Graphpad version 5 software.

2.7. Statistical Analysis

The data obtained from this study were subjected to analysis of variance based on the general linear model using program SPSS (Statistical Package for Social Science, version 16.0). The statistical significance of differences among the means of the control and different treatments was determined by a One-way analysis of variance. A P value of < 0.05 was considered significant.

3. Result

3.1. Fatty Acid-binding Protein's Induced Goose Fatty Liver by Overfeeding

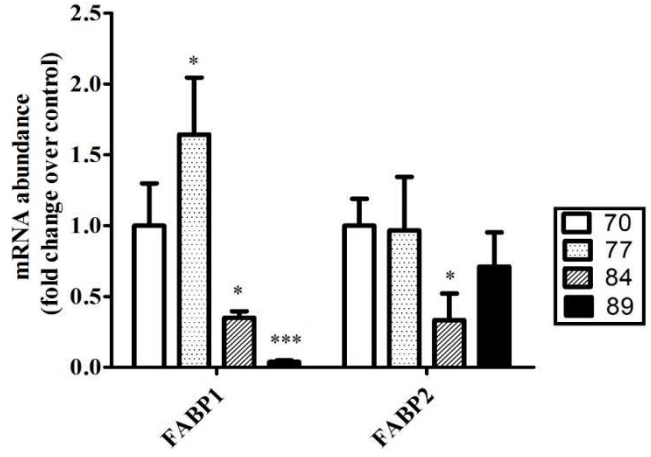

Figure 1. Histogram showing the up-regulation of fatty acid-binding protein 1, 2 in the livers of the overfed vs. normal fed geese. The mRNA abundance was determined by qPCR. The control group consists of the geese that were normally fed, while overfeeding group consists of the geese that were overfed for 0, 7, 14, and 19 days (i.e., the overfed geese at 70, 77, 84 and 89 days of age). The mRNA abundance in the overfeeding group was presented as fold change over the control group. N=6. *, *** denote P<0.005, 0.001 vs. control, all data are presented as means ± SEM.

The expression levels of fatty acid-binding proteins genes were determined by quantitative PCR. qPCR data revealed that, in the treated groups compared to the control, the expression levels of *FABP2* gradually decreased with time of overfeeding in the livers of the overfed geese, while the FABP1 was decreased after one week of overfed. In addition, the expression levels of geese *FABPs1* mRNA in the overfeeding group at 89 days was the lowest compared to control group Figure 1.

3.2. FABPs1/2 Genes Regulation by Fatty Liver-related Factors in Goose Primary Hepatocytes

Goose primary hepatocytes were treated with high levels of glucose, fatty acid, and insulin. qPCR data indicated that the expression of *FABP1/2* were inhibited by high levels of glucose (25-50 mM) and insulin (50-100mM) for *FABP1* in goose hepatocytes Figure 1/2. However, *FABP2* was not significantly (p 0.05) regulated by insulin Figure 2. In addition, for the fatty acid treatment, the expression levels of *FABP1/2* were not significantly changed in Palmitate (0.25 or 0.5mM) treatment Figure 4. Furthermore, the expression of *FABP2* was induced by high levels of Oleate (0.5 mM), while *FABP1* was not affected Figure 5.

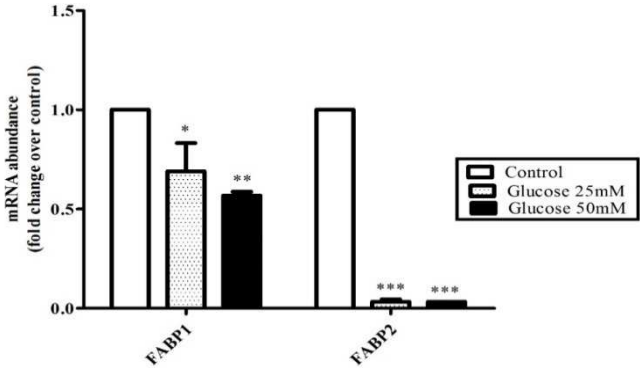

Figure 2. Histogram illustrating the mRNA expression of fatty acid-binding protein 1, 2 in goose primary hepatocytes treated with glucose vs. without glucose. The expression of the genes was determined by quantitative PCR. Primary hepatocytes isolated from goose embryos at 21 days of pre-hatch and then were treated with 25 mM, and 50 mM glucose in serum-free media, while primary hepatocytes untreated with glucose were used as control. The average mRNA abundances of genes in hepatocytes were treated with different levels of glucose and then presented as fold change over the control. N=3. *, **, *** denote represent P<0.005, 0.01, 0.001 vs. control, respectively. All data are presented as means ± SEM.

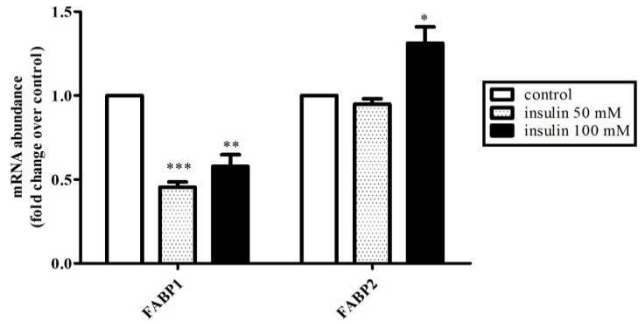

Figure 3. Histogram showing the mRNA expression of fatty acid binding protein 1, 2 in goose primary hepatocytes treated with glucose vs. without insulin. The expression of the genes was determined by quantitative PCR. Primary hepatocytes were isolated from goose embryos at 21 days of pre-hatch were treated with 50 mM, and 100 mM insulin in serum-free media, while primary hepatocytes untreated with insulin were used as control. N=3. The average mRNA abundances of genes in hepatocytes were treated with different levels of insulin and then presented as fold change over the control. N=3. *, **, *** denote represent P<0.005, 0.01, 0.001 vs. control, respectively. All data are presented as means ± SEM.

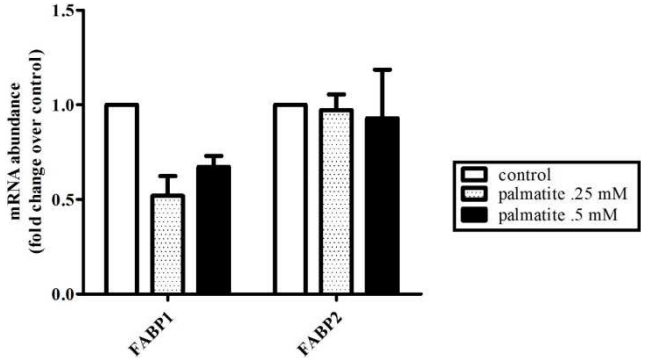

Figure 4. Histogram illustrating the mRNA expression of fatty acid binding protein 1, 2 in goose primary hepatocytes treated with Palmitate vs. without Palmitate. The expression of the genes was determined by quantitative PCR. Primary hepatocytes isolated from goose embryos at 21 days of pre-hatch were treated with 0.25 and 0.5 mM Palmitate in complete cell culture media, while the primary hepatocytes were used as an untreated Palmitate control. The average mRNA abundances of genes in hepatocytes were treated with different levels of Palmitate and presented as fold change over the control. N=3. All data are presented as means ± SEM.

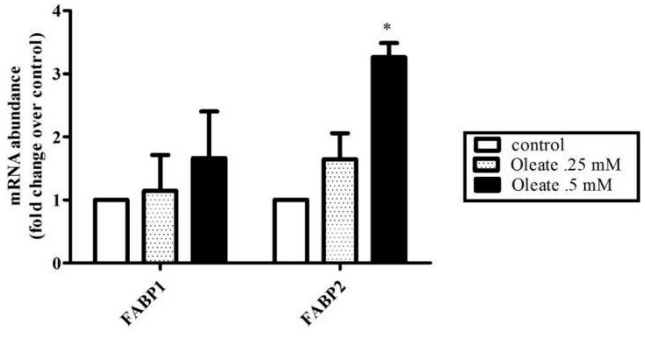

Figure 5. Histogram demonstrating the mRNA expression of fatty acid binding protein 1, 2 in goose primary hepatocytes treated with Oleate vs. without Oleate. The expression of genes was determined by quantitative PCR. Primary hepatocytes isolated from goose embryos at 21 days of pre-hatch were treated with 0.25 Mm and 0.50 mM Oleate in complete cell culture media, while untreated primary hepatocytes were used as control. The average mRNA abundances of the genes in hepatocytes treated with different levels of Oleate are presented as fold change over the control. N=3. * denote P< 0.005 vs. control. All data are presented as means ± SEM.

4. Discussion

The liver is considering as an important organ for lipid metabolism *in vivo* experiments. In general, increasing of the fatty acids are positively correlated with the development of obesity and insulin resistance, while paradoxically, adipocyte/macrophage *FABP* deficient mouse models were more insulin sensitive [14]. Fatty acid binding protein 1 (*FABP1*) is presumably mediate the intracellular movement and metabolism of fatty acids [15]. A previous study has suggested that *FABP1* protein exist at a low concentration in the kidney of the genetically hypertensive rats, the study was based on a binding assay measuring the capacity of a soluble extract to bind radiolabeled fatty acid [16]. The present study indicates that the expression levels of FABP1, 2 in liver tissue of both control and treatment were decreased after two weeks of overfeeding period. These finding suggest that FABP1, 2 may play an important role in fatty liver development with high carbohydrate diet. In addition, the results noted that the high carbohydrate diet may has an encourage effect for the fatty liver functions and metabolism in the first stage of overfeeding. Furthermore [17] illustrated that the L-FABP play a crucial role in the oxidative tissues of the liver. The abovementioned findings suggest that the expression of *FABP2* gene is hypersensitive inhibited by the fatty liver-associated factors, such as hyperglycemia and other factors causing the inhibition of the gene in goose fatty liver. Although it is known that *FABP1/2* can be regulated by hormones (e.g., insulin) [18] and a variety of fatty acids (e.g., arachidonic acid, and docosahexaenoic acid) [19-22].

In SHRSP rats, the binding activities of Palmitate, stearate, Oleate, linoleate, and arachidonate are increased in early age (5 weeks), while the contrary is observed at the age of (40 weeks) [16]. In the goose hepatocytes treated with Palmitate and Oleate the current study, revealed that there was no significant effect. Moreover, *FABP1* treated with high levels of glucose and insulin, the expression levels were affected and continuasly reduced while, the level of glucose and insulin increased. [23] suggested that the fatty acid binding protein 1 is more sensitive for glucose and insulin medium, while *FABP2* expression also have positive effect in insulin and it may reflect that on the role of metabolic response to dietary fat [23]. In respect to RT-PCR analysis the present study showed that the *FABP2* expression was significantly decreased in glucose treatment, as well as the liver of overfeeding goose. The formation mechanism of fatty liver in goose is complicated and the fatty liver traits cannot be selected or measured in living organisms. Therefore, further studies may be necessary to investigate the fatty liver in goose and other species of birds.

5. Conclusion

The differential expression analysis showed that the expression levels of *FABP1/2* were highly significant affected by overfeeding and embryonic primary hepatocytes treated with glucose and insulin in lands geese fatty liver. These findings indicated that the expression of *FABP2* gene was sensitive to the fatty liver-associated factors, such as hyperlipidemia in goose fatty liver. In consistence with previous studies, compared to normally fed Landes geese, the overfed geese developed severe fatty liver after 19 days of overfeeding.

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