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RESEARCH ARTICLE

Glucose and insulin modulate inflammation and glycolipid metabolism genes in goose muscle via *IGFBP2* during fatty liver development

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Abstract

Muscle, the largest tissue in geese, is crucial for developing goose fatty liver. Investigating the influence of Insulin-like Growth Factor Binding Protein 2 (*IGFBP2*) on muscle during fatty liver development is crucial for understanding its regulatory mechanisms. In this study, twenty-four 70-day-old male Landes geese were divided into two groups: an overfeeding group and a control group (n = 12 each). Pectoral muscle samples were collected at 82 and 94 days of age. Additionally, twenty-four 10-day-old geese were divided into a glucose injection group and a saline control group (n = 12 each), with pectoral muscle samples collected 2 hours post-injection at 17 days of age. Goose primary myocytes were treated with glucose (0, 25, 50, and 100 mmol/L) or insulin (0, 5, 10, and 20 nmol/L) and transfected with an *IGFBP2* overexpression vector or an empty vector. Results demonstrated that overfeeding, glucose injection, 50 and 200 mmol/L glucose treatment, and 10 and 20 nmol/L insulin treatment significantly inhibited the expression of *IGFBP2* in both muscle and cell samples ($P < 0.05$). Overexpression of *IGFBP2* increased the expression of LOC106030908, FGB, and LOC106040417, while inhibiting PLPP4, PTGS2, IL6, and LOC106041919 ($P < 0.05$). Notably, gene expression showed an inverse pattern in the muscles of overfed geese. Additionally, glucose and insulin upregulated LOC106041919 and LOC106040417 while downregulating LOC106030908 in goose myocytes ($P < 0.05$). These results suggest that glucose and insulin modulate inflammation and glycolipid metabolism genes in goose muscle via *IGFBP2* during fatty liver development, providing new molecular insights.

Keywords: food nutrition improvement, lipotoxicity, metabolic regulation, nutrient metabolism, poultry physiology

INTRODUCTION

Goose fatty liver is a type of steatosis caused by short-term artificial overfeeding with carbohydrate-rich diets (Zhu et al., 2011). The condition develops when liver fat production exceeds its ability to process triacylglycerol (TG) and to break down fatty acids. The liver accumulates excessive TG due to its inability to process them properly (Hermier et al., 1991; Wei et al., 2023). The development of fatty liver in mammals depends heavily on insulin resistance (IR). The liver produces excessive fat due to elevated blood glucose and insulin levels caused by IR (Knobler et al., 1999). The development of fatty liver in geese occurs through IR, which leads to elevated blood glucose and insulin levels in overfed birds compared to normally-fed birds (Geng et al., 2015). Plasma insulin levels in Landes geese and Sichuan white geese have been reported to significantly rise after overfeeding (Han et al., 2008).

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Insulin and glucose are major regulators of nutrient partitioning during goose fatty liver formation. In hepatocytes, insulin promotes glucose uptake, glycogen synthesis, and lipogenesis, while inhibiting lipolysis (Szablewski, 2024). In skeletal muscle, insulin also enhances glucose utilisation and supports anabolic processes, including fatty acid synthesis (Ritter et al., 2015). Under overfeeding conditions, excess dietary carbohydrate increases glucose availability. Glucose that is not oxidised for energy or stored as glycogen can enter glycolysis to generate acetyl-CoA, a key precursor for fatty acid synthesis (Kumari et al., 2024). These fatty acids are subsequently esterified with glycerol to form triacylglycerol, which may accumulate in tissues. Therefore, changes in glucose and insulin levels are likely to be closely associated with lipid deposition and metabolic adaptation during the development of goose fatty liver. The system in glucose and lipid metabolism as well as inflammation in animals involves insulin-like growth factor binding proteins (IGFBPs), insulin-like growth factors (IGFs), and their receptors (IGFRs). The IGFBP2 protein of the *IGFBPs* family plays an important role in producing and secreting adipocytes after they reach maturity (Wilhelm et al., 2013). The protein supports growth processes and facilitates glucose absorption (Pliszka et al., 2006). In poultry, the exact role and operational mechanisms of *IGFBP2* remain poorly understood, especially in the context of fatty liver development.

For energy intake, tissue from livestock and poultry skeletal muscles are considered the most prominent in their bodies, which a large proportion is utilized. The involvement of muscle in lipid metabolism has great influence in the development of fatty liver disease. This study examines the role of *IGFBP2* in the development of fatty liver in geese, in which the expression of *IGFBP2* in diet-restricted versus overfed geese is investigated. In the study, some geese were glucose-injected, and primary goose muscle cells were treated then with glucose and insulin. The effects of *IGFBP2* overexpression in primary goose hepatocytes on the secretion of insulin-like growth factor, glucose, lipid metabolism, and inflammatory cytokines are also determined. This study is able to fill existing gaps in knowledge about the role and mechanism of *IGFBP2* in goose fatty liver, of which the scope of poultry physiology and pathology will be expanded.

Earlier studies show that *IGFBP2* controls fat production and insulin response mainly in the liver and fat tissue (Wilhelm et al., 2013; Stanley et al., 2021). In poultry, skeletal muscle is the main site for glucose use, handling more than 70% of insulin-driven glucose uptake when fed (Rui, 2014). Unlike in the liver and fat, where *IGFBP2* affects IGF-1 activity, in muscle it may act through other routes that control how muscle cells take in glucose and store it as glycogen (Norton et al., 2022). This muscle-specific role has not been studied during fatty liver development in geese.

MATERIALS AND METHODS

Overfeeding experiment

A total of 24 healthy male Landes geese, 70 days of age and with similar body weights, were used in the overfeeding experiment. After a 5-day preparatory period, the birds were randomly assigned to a control group (n = 12) and an overfeeding group (n = 12). Geese were housed individually in a semi-open shed under natural light, with free access to water throughout the experiment. The overfeeding procedure followed our previous protocol (Osman et al., 2016). Briefly, the daily feed allowance was gradually increased from 100 g to 300 g during the preparatory period. During the formal overfeeding period, geese received 500 g feed per day in three meals during the first 5 days, followed by 1,200 g per day in five meals for the remaining period. The diet consisted of cooked maize supplemented with 1% vegetable oil and 1% salt. Pectoral muscle samples were collected from the same anatomical site according to the experimental schedule. Before tissue collection, birds were fasted overnight but allowed free access to water. The following morning, geese were weighed and euthanized with CO_2. The excised pectoral muscle samples were briefly rinsed with physiological saline to remove residual blood, blotted dry, placed directly into sterile cryotubes without any added solution, snap-frozen in liquid nitrogen, and stored at −80 °C until further analysis.

Glucose injection experiment

A separate cohort of 24 healthy male Landes goslings, 10 days of age and weighing 210 ± 10 g, was used for the glucose injection experiment. The birds were reared at the National Waterfowl Gene Bank (Taizhou, Jiangsu, China) in a closed house under continuous light provided by thermostatically controlled infrared lamps, with the ambient temperature maintained at 28–30 °C and relative humidity at 60%–65%. The diet consisted of 40% maize, 20% broken rice, 14% rice bran, 10% soybean meal, 4.7% fish meal, 10.5% wheat bran, and 0.3% vitamin premix. The goslings were randomly assigned to a saline-treated control group (n = 12) and a glucose-treated group (n = 12). D-(+)-glucose powder was dissolved in physiological saline to prepare a glucose solution at a final concentration of 0.8 g/mL. The glucose solution was administered by

intraperitoneal injection into the lower abdomen at a dose of 2 g/kg body weight on alternate days. Accordingly, the four injections were given on days 10, 12, 14, and 16 of age. Birds in the control group received physiological saline on the same schedule. Two hours after the fourth injection, the goslings were euthanized with CO_2 in accordance with the approved animal protocol. Pectoral muscle samples were collected from the same anatomical site within 1 h after euthanasia, briefly rinsed with physiological saline to remove surface blood, blotted dry, placed directly into cryotubes without any added solution, snap-frozen in liquid nitrogen, and stored at −80 °C until further analysis. The animal procedures were approved by the Animal Care and Use Committee of Yangzhou University (approval no. SYXK(Su)2021-0026).

Isolation and treatment of primary goose pectoral muscle cells

Goose primary pectoral muscle cells were extracted and seeded into culture plates according to methods described in a previous study (Osman et al., 2016). Upon reaching approximately 70% confluence, the cells were cultured for 12 hours in a complete culture medium composed of high-glucose DMEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (100 IU/ml), and 10 μL of epidermal growth factor (EGF, 20 ng/ml). Following this, the cells were treated with various concentrations of glucose (0, 25, 50, and 100 mM) or insulin (0, 5, 10, and 20 nM) for 14 hours before being collected for further analyses. An *IGFBP2* overexpression vector was constructed based on the gene sequence of *IGFBP2* (GenBank accession number XM_013196707.2). A control plasmid was generated using the pcDNA3.1 vector backbone driven by a CMV promoter. Both the *IGFBP2* overexpression plasmid and the empty pcDNA3.1 control plasmids were introduced into primary goose pectoral muscle cells for subsequent studies.

RNA isolation, cDNA preparation, and quantitative PCR

Total RNA was extracted from pectoral muscle cells or tissue and converted to complementary DNA (cDNA) by reverse transcription (Li et al., 2023). Primer sequences for *IGFBP2* and its downstream targets were designed using Primer Premier 5.0 (Table 1). Quantitative real-time PCR (qPCR) was performed using *β-actin* (accession XM_013174886.1) as the reference gene. Each 20 μL reaction contained 10 μL SYBR® Premix Ex Taq (2×), 0.4 μL of each primer, 2.0 μL cDNA, and 6.8 μL double-distilled water. Amplification and melt-curve analyses were carried out with QuantStudio™ Design & Analysis Software v1.5.2 (Applied Biosystems). All reactions produced a single melt peak per amplicon. Representative melt curves are shown in Supplementary Figure 1 and mean ± SD melting temperatures (Tm) are listed in Supplementary Table 1.

The PCR amplification protocol began with an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 65 °C for 30 s. A melt curve test was utilized to verify that the PCR amplification was precise and specific, using the following temperature steps: incubations at 95 °C for 15 seconds, 60 °C for 1 minute, followed by 95 °C for another 15 seconds. Quantification of gene expression was performed using the comparative $2^{-\Delta\Delta Ct}$ approach.

Statistical analysis

Data from gene expression experiments in pectoral muscle and primary goose myocytes were analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Comparisons between two groups were performed with two-sided unpaired t-tests using Welch's correction These comparisons included overfed geese versus control geese at each sampling point, glucose-injected geese versus saline-injected controls, and IGFBP2-overexpression versus empty-vector control cells. For dose-response experiments in primary myocytes, namely glucose treatment (0, 25, 50, and 100 mM) and insulin treatment (0, 5, 10, and 20 nM), one-way ANOVA followed by Dunnett's multiple-comparison test versus the corresponding 0 mM or 0 nM group was applied, and multiplicity-adjusted P values were reported. When multiple genes were compared between two conditions, multiple unpaired t-tests with Welch's correction were performed with false discovery rate (FDR) control at Q = 5%. Data are presented as mean ± SD, and significance was set at $P < 0.05$, unless otherwise stated. Biological replicates represented independent animals or independent primary myocyte cultures prepared from different 23-day-old goose embryos, whereas technical replicates represented repeated qPCR measurements from the same sample, which were averaged before statistical analysis.

Table 1. Primer information for gene expression analysis

Gene	Primer sequences（5'→3'）	Product size (bp)	Accession number
IGFBP2	F:GGGAGAAGGTGAATGAGCAG R:GAGGGAGTAGAGGTGCTCCA	194	XM_013196707.2
MAP3K7CL	F: CAGCCTCTGCCTCCTTGTC R: GCTTCAGCGTTCGGTTCTC	226	XM_048071423.1
SLC40A1	F:CTGGGGAGATCGTATGTGGC R:AGGATGTCTGGGCCACTTTG	179	XM_048058484.1
STAT4	F:GCACGCCTTTTGTTCGGTAT R:TCCATTGGGACATACTCAGCAC	200	XM_048058203.1
LOC106041089	F:CCCAGCCAGGCGTGATATTC R:TGATGCTTGAAGGGAGCAGG	143	XM_013189339.2
IL6	F:CGACGATAAGGCAGATGGTGATA R:ACAGCCCTCACGGTTTTCTC	172	XM_048070285.1
LOC106037025	F:CAGAAGACAGGAGGTGAGCC R:GTCATCCTGTGCCTCCTATGG	115	XM_013182731.2
LOC106041041	F:CAGCTTCGTGACCGACTACT R:CCTTCCTTGTGATGAACACGAC	154	XM_013189266.2
LOC106041919	F:CAGAGAAGTGTGCCTGGACC R:GCTTGGTGTTTGCCTTGTCC	80	XM_013190619.2
LOC106038554	F:ACGAGCAATGTGACCGTCTT R:ACCCAGTGTTTTACCCAGCC	238	XM_048070544.1
LOC106042256	F:TGCAAACTGCGGAAGATAATGA R:GGCAAGGGAAATGAATCCAGC	210	XM_013191039.2
RSAD1	F: GCGTCAACTACCACTTCACC R: AATTTTCTCCATTCCCGCCTCC	140	XM_013172803.2
FGB	F: ACCCAAACAGACTACTGCCG R: TGAATCAAAGTCCAGCCTCCA	197	XM_013175323.2
CD9	F: TTTAAGGAGTGGCGCTGTGG R: TTAGTGCCTCCTTTGACGGG	108	XM_013196002.2
LOC106030908	F: CAGATTGAGCTCTGGTGGCA R: GAGACAGTGGCGTTCCTGG	128	XM_013172955.2
LCAT	F: GTGTCCTGGCATCTGGTGATA R: GCTTGTAGTCCCGGTAGGTG	177	XM_013176745.2
LOC106040417	F: CATGGATGGACGGTGACAGG R: CCACCAGCTTAGTGTACGCA	157	XM_048051490.1
PTGS2	F: ATCCTTGCTGCTCAAACCCA R: CCATGTGAAGAATTCCGGTGTTG	131	XM_048058960.1
PLPP4	F: TTCGGCGGACAGACAAGACT R: AAATCGGGACGCGGTCTTC	121	XM_048058247.1
PLA2G4A	F: GCAGGATCATATCCCGGCG R: GCTTCCAGCACCTCTGGG	108	XM_048058954.1
B-ACTIN	F: CAACGAGCGGTTCAGGTGT R: TGGAGTTGAAGGTGGTCTCG	132	XM_013174886.1

RESULTS

Comprehensive analysis of the effects of overfeeding, glucose, insulin, and IGFBP2 overexpression on cytokine gene expression and glycolipid metabolism in goose muscle and myocytes

This study systematically investigated the effects of overfeeding, glucose, insulin, and the impact of *IGFBP2* overexpression on genes associated with glycolipid metabolism and cytokines in goose muscles and primary myocytes. In the initial phase of the study, *IGFBP2* mRNA expression in the pectoral muscle of 70-day-old Landes geese was markedly decreased after 12 days of overfeeding in comparison to the control group ($P < 0.05$), as shown in Figure 1, signifying that early overfeeding induces a significant reduction in *IGFBP2* expression. Nonetheless, following 24 days of excessive eating, the mRNA expression levels were comparable to those of the control group, indicating a time-dependent phenomenon wherein *IGFBP2* expression stabilizes with extended periods of overfeeding. This pattern indicates a particular regulatory response in muscle metabolism during the initial phases of overfeeding.

The second experiment showed that giving 10-day-old geese glucose decreased *IGFBP2* expression in the pectoral muscle significantly compared to controls that were given saline ($P < 0.05$, Figure 2). The significant decrease in *IGFBP2* suggests that glucose exerts a direct influence on the regulation of gene expression associated with muscle metabolism.

Moreover, glucose lowered *IGFBP2* mRNA levels at 50, 100, and 200 mmol/L compared with 0 mmol/L. Insulin reduced *IGFBP2* at 10 and 20 nmol/L, while 5 nmol/L had no effect (one-way ANOVA with Dunnett's test; adjusted $P < 0.05$; Figures 3A and 3B).

To examine the effect of increased *IGFBP2* expression, goose primary myocytes were transfected with an *IGFBP2* overexpression vector or the corresponding empty vector. Quantitative PCR showed that *IGFBP2* mRNA levels were significantly higher in the overexpression group than in the control group ($P < 0.01$; Figure 4), confirming successful overexpression. We then assessed the expression of genes related to glycolipid metabolism and inflammatory cytokine signalling. Overexpression of *IGFBP2* significantly increased the expression of LOC106030908 (*HKDC1*), LOC106040417 (*LPL*), and *FGB*, while significantly decreasing the expression of *PLPP4*, LOC106038123 (*IL17F*), *PTGS2*, *IL6*, and LOC106041919 (*IL8*) ($P < 0.05$; Figure 5).

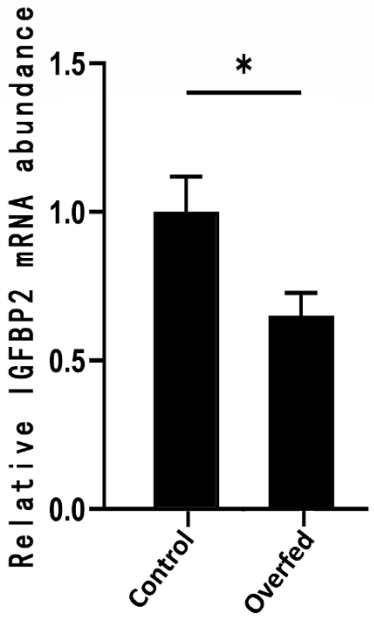

Figure 1. Effect of overfeeding on *IGFBP2* mRNA levels in the pectoral muscle of Landes geese. Bars represent mean ± SD. Unpaired t-test with Welch's correction; *$P < 0.05$ vs. control ($n = 12$).

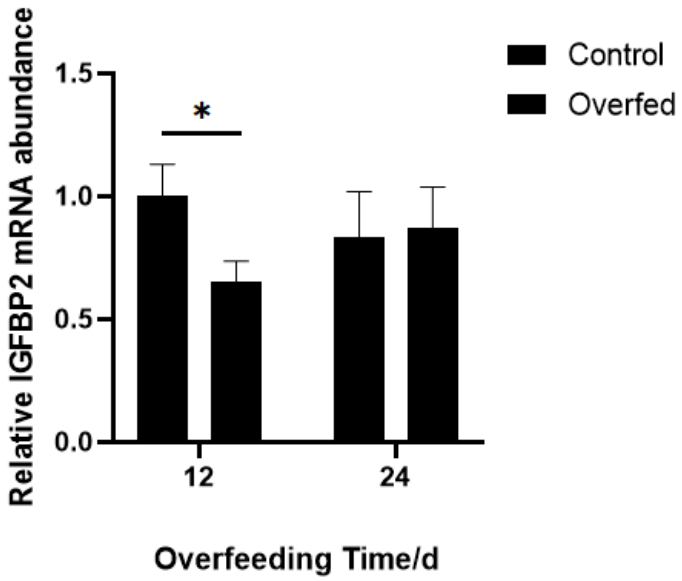

Figure 2. Effect of glucose injection on *IGFBP2* mRNA levels in the pectoral muscle of Landes geese. Bars represent mean ± SD. Unpaired t-test with Welch's correction; *$P < 0.05$ vs. control ($n = 12$).

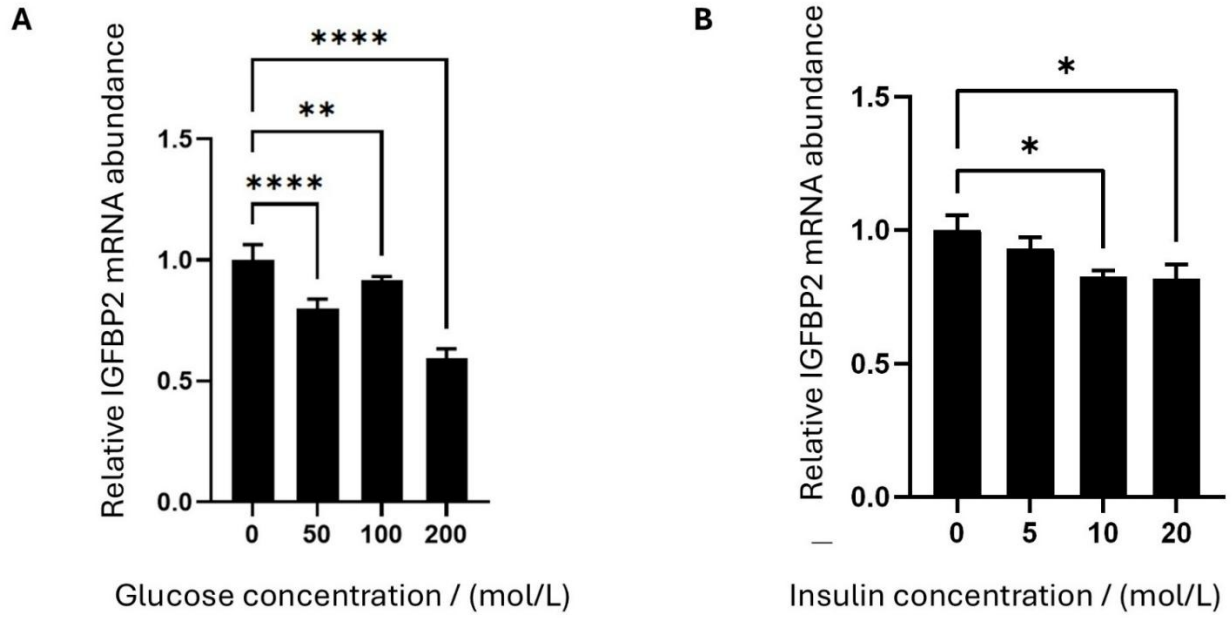

Figure 3. Effect of glucose (A) and insulin (B) on *IGFBP2* mRNA in goose primary myocytes. Bars represent mean ± SD. One-way ANOVA with Dunnett's multiple comparisons vs. 0; *, **, **** indicate adjusted $P < 0.05$, < 0.01, and < 0.0001, respectively ($n = 6$).

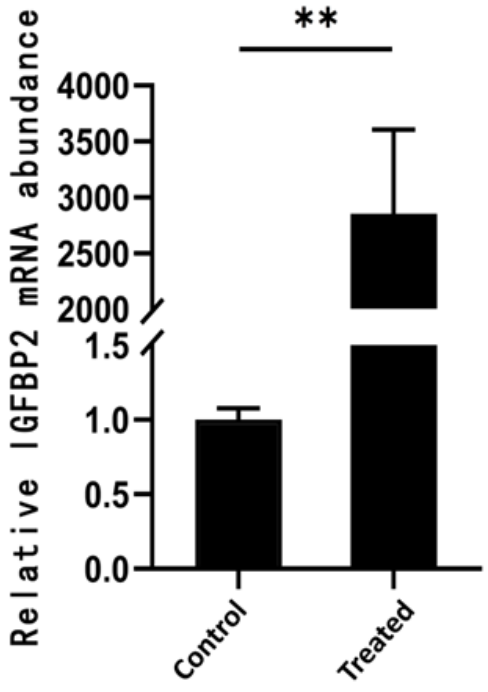

Figure 4. *IGFBP2* mRNA levels in goose primary myocytes transfected with the overexpression vector compared with the empty vector. Bars represent mean ± SD. Unpaired t-test with Welch's correction; **$P < 0.01$ vs. control ($n = 6$).

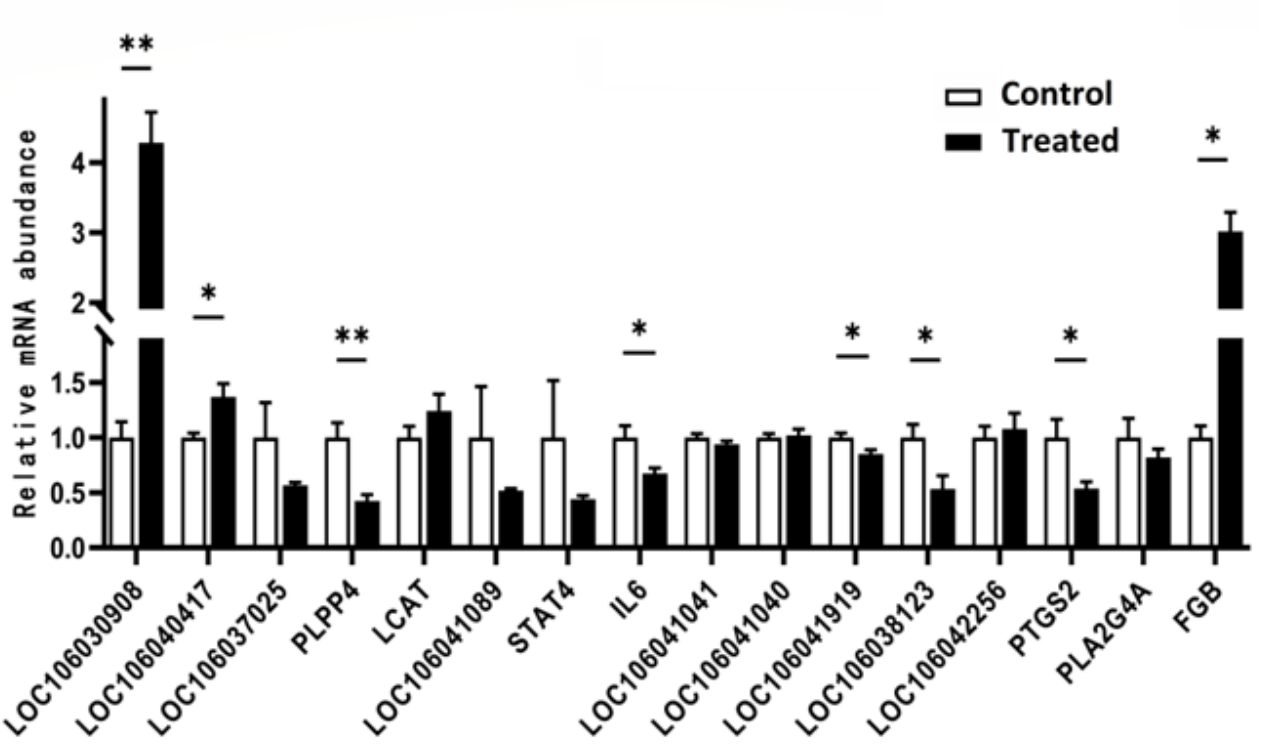

Figure 5. Effect of *IGFBP2* overexpression on genes involved in glycolipid metabolism and cytokine expression in goose primary myocytes. Bars represent mean ± SD. Multiple unpaired t-tests (Welch) with FDR control (Q = 5%); asterisks indicate significance after FDR adjustment (n = 6).

The study also examined the impact of overfeeding on the identical set of genes. After 12 days of overfeeding, the expression levels of genes such as *PLPP4*, *IL6*, and *LOC106041919* were markedly elevated ($P < 0.05$), indicating an inflammatory response linked to excessive food intake. The expression levels of *LOC106030908*, *LOC106040417*, *PTGS2*, and *FGB* were considerably diminished ($P < 0.05$, Figure 6), indicating a disruption in normal glycolipid metabolism during the progression of fatty liver.

In a similar manner, glucose treatment of goose primary myocytes resulted in the suppression of *LOC106030908* and *PLPP4* while markedly enhancing the expression of *LOC106040417*, *IL6*, *LOC106041919*, and *PTGS2* ($P < 0.05$, Figure 7). These findings substantiate the notion that heightened glucose levels, akin to overfed, incite inflammatory responses and modify the control of metabolic pathways. A similar pattern was noted with insulin administration (20 nmol/L), which reduced *LOC106030908* and activated *PLPP4*, *LOC106041919*, and *LOC106040417* ($P < 0.05$, Figure 8). This underscores insulin's role in regulating glycolipid metabolism and the expression of inflammatory genes in muscle cells.

Effect of overfeeding on igfbp2 gene expression in the pectoral muscle of landes geese

The mRNA expression levels of *IGFBP2* in the pectoral muscle of 70-day-old Landes geese, after the 12th and 24th day of overfeeding, were measured using qRT-PCR. Results indicate that, compared to the control group, there was a significant reduction in *IGFBP2* mRNA expression after 12 days of overfeeding ($P < 0.05$). However, after 24 days of overfeeding, there was no significant difference in *IGFBP2* expression between overfed and control groups. Figure 1 illustrates the pronounced reduction in *IGFBP2* expression by day 12, which then stabilizes by day 24, with expression levels comparable to those of the control group. The findings suggest a temporal component in the effect of overfeeding on the *IGFBP2* gene expression; the majority of the downregulation occurs early in the overfeeding period, with only a modest decrease observed during the later stages.

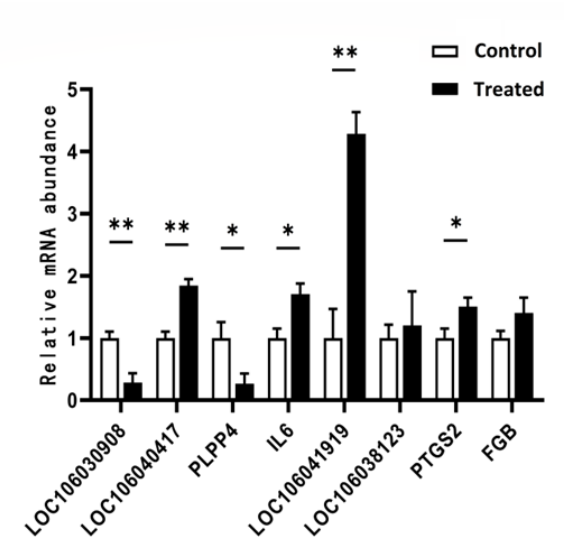

Figure 6. Effect of 12-day overfeeding on the same gene set in pectoral muscle. Bars represent mean ± SD. Multiple unpaired t-tests (Welch) with FDR control (Q = 5%); asterisks indicate significance after FDR adjustment (n = 12).

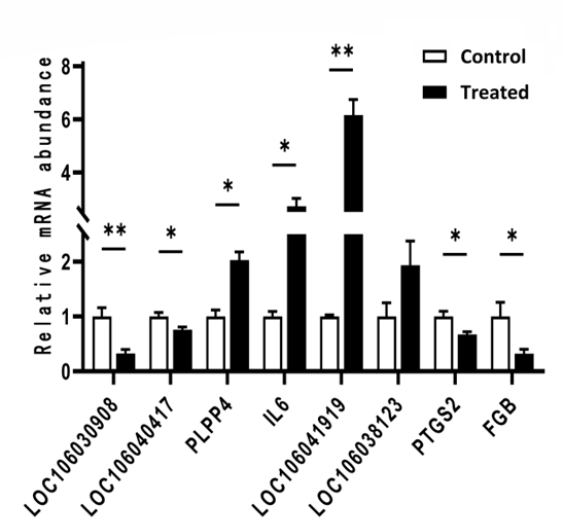

Figure 7. Effect of glucose treatment on glycolipid metabolism and cytokine gene expression in goose primary myocytes. Bars represent mean ± SD. Multiple unpaired t-tests (Welch) with FDR control (Q = 5%) (n = 6).

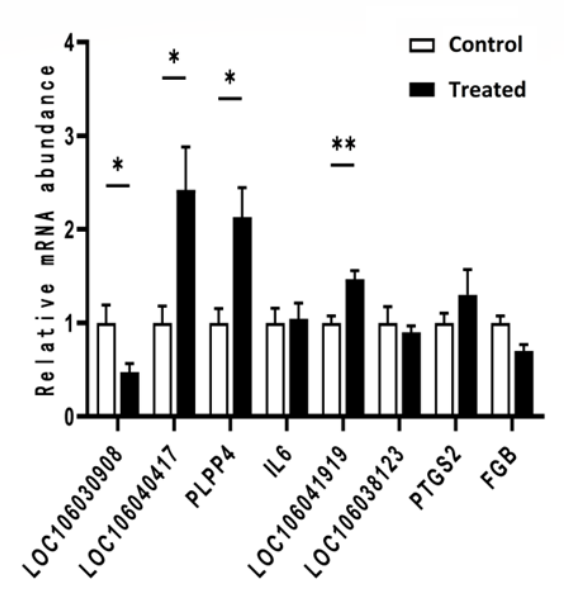

Figure 8. Effect of insulin treatment on the same gene set in goose primary myocytes. Bars represent mean ± SD. Multiple unpaired t-tests (Welch) with FDR control (Q = 5%) (n = 6).

DISCUSSION

Regulation of IGFBP2 gene expression in muscle during goose fatty liver development

Muscle tissue is known to be the primary site for energy metabolism in the body and plays a critical role in the development of goose fatty liver (Rui, 2014). Prior studies reported that excessive lipid accumulation in muscle tissue contributes to lipotoxicity during non-alcoholic fatty liver disease (NAFLD), which subsequently induces oxidative stress, apoptosis, and inflammation (Svegliati-Baroni et al., 2019). These processes will impair muscle cell responsiveness to insulin, which further promotes IR. Then, this will further elevate blood glucose and insulin levels, which in turn fosters hepatic lipofibrosis and steatosis, exacerbating the progression of fatty liver disease. Furthermore, increased hepatic lipogenesis and lipid secretion will raise plasma lipid concentrations, which further intensifying muscle lipid infiltration and establishing a self-perpetuating cycle. Therefore, in the context of NAFLD, progressive loss of muscle activity will lead to excessive lipid deposition and insulin resistance, as the two central components (Norton et al., 2022).

The findings of this study found that there was a significant reduction in *IGFBP2* mRNA expression in the pectoral muscle of Landes geese after 12 days of overfeeding, suggesting that the expression pattern of *IGFBP2* in muscle tissue aligned with patterns previously observed in the liver and adipose tissue as fatty liver disease develops in geese (Li et al., 2023).

Hepatic insulin action promotes glucose uptake, stimulates glycogen synthesis, and enhances lipogenesis in hepatocytes (Hodson and Gunn, 2019). In the study by Hodson and Gunn (2019), the role of insulin in adipose tissue involves facilitating cellular glucose uptake and promoting the esterification of free fatty acids into triglycerides. Insulin action in muscular tissue also stimulates fatty acid production (da Silva Rosa et al., 2020). Furthermore, glucose serves as a major source of energy. Following periods of high intake, geese consume substantial amounts of carbohydrates, which are subsequently converted into glucose. Besides being oxidized for converted to energy or stored as glycogen in the liver and muscle tissues, the excess glucose undergoes glycolysis to produce acetyl-CoA, a key precursor for the synthesis of fatty acids (Felix et al., 2021). These fatty acids are then esterified with glycerol to produce triglycerides, which are accumulated in the liver and adipose tissue. The above findings indicate that fluctuations in glucose and insulin levels within the body occur in response to shifts in nutrient and energy levels, potentially mediating the regulation of animal physiological functions in response to these changes.

Inhibition of IGFBP2 gene expression in muscle by glucose and insulin

In the course of goose fatty liver development, blood glucose and insulin levels rise significantly (Zambonelli et al., 2016), and systemic IR is observed (Geng et al., 2016). In this study, glucose injection and treatments with varying concentrations of glucose and insulin resulted in a substantial reduction in *IGFBP2* mRNA expression in goose muscle and primary muscle cells. Thus, the lower expression of *IGFBP2* in muscle during the development of goose fatty liver may be partially explained by the elevated levels of blood glucose and insulin. Similarly, in hyperglycemic mouse models, plasma *IGFBP2* levels correlate with IR, and *IGFBP2* is considered a biomarker of insulin sensitivity (Han et al., 2010). During IR, elevated insulin levels lead to reduced *IGFBP2* expression in tissues and decreased release of *IGFBP2* into bloodstream. The relationship between insulin and *IGFBP2* expressions has been documented in several studies. For instance, hepatic *IGFBP2* gene expression is significantly upregulated during fasting in mice (Ko et al., 2012), whereas insulin suppresses *IGFBP2* gene expression in myocytes and cardiac tissue (Ko et al., 2012). As suggested by Orlowski et al. (1990), the inhibitory effect of insulin on *IGFBP2* expression may be mediated through the PI3K/mTOR signaling pathway. These findings match the known control of *IGFBP2* by diet and hormones. In hepatocytes, insulin lowers *IGFBP2* expression, while fasting increases it through PPARα-dependent pathways (Böni-Schnetzler et al., 1990; Kang et al., 2016). In birds, the main ~30 kDa IGFBP rises during fasting, reflecting a catabolic state (Morishita et al., 1993). Overfeeding in geese disrupts glucose–insulin balance and causes systemic IR(Geng et al., 2016). Our muscle and myocyte results fit this pattern, showing that high glucose and insulin reduce *IGFBP2* expression in skeletal muscle. This response is similar to human and mouse data linking low *IGFBP2* levels with IR and NAFLD (Haywood et al., 2020; Boughanem et al., 2021).

The mechanism behind this effect likely involves insulin-responsive signaling. Pathways such as PI3K/AKT–FOXO are known to suppress *IGFBP2* transcription in metabolic tissues (Böni-Schnetzler et al., 1990; Kang et al., 2016). The reduction in *IGFBP2* after glucose or insulin exposure in goose myocytes likely reflects this conserved insulin-dependent repression. In geese, overfeeding-related hyperinsulinemia may lower muscle *IGFBP2* at early stages, while later recovery in vivo could signal adaptation to sustained nutrient load. This pattern aligns with our data and previous avian studies showing fasting–refeeding shifts in IGF/IGFBP

profiles (Morishita et al., 1993). Work on *ACSBG2* in geese also shows that feeding status affects steroid hormone and cell-adhesion gene expression in liver, which may influence muscle *IGFBP2* through endocrine or paracrine signals (Wang, 2024).

Consistent with the observed reduction in muscle *IGFBP2* expression after 12 days of overfeeding, Li et al. (2012) also previously reported a significant suppression of *IGFBP2* expression in the liver of Landes geese after both 12 and 24 days of overfeeding. Based on these results, it supports the conclusion that both in vivo conditions, such as glucose injection, and in vitro exposure to elevated glucose or insulin levels suppress *IGFBP2* expression in muscle cells, thereby highlighting the metabolic sensitivity of *IGFBP2* to dietary and hormonal changes.

Overall, the results indicate that during the progression of NAFLD, IR in peripheral tissues, especially in muscle, contribute to elevated blood glucose and insulin levels. This, in turn, results in the suppression of *IGFBP2* expression in both muscle and liver. These findings also suggest that overfeeding could decrease muscle *IGFBP2* expression, potentially through mechanisms like hyperglycemia and hyperinsulinemia. This reduction in *IGFBP2* expression may, in turn, affect the expression of downstream genes it regulates, playing a role in the development of fatty liver in geese.

Biological effects and mechanisms mediated by *IGFBP2* gene in muscle during goose fatty liver development

The IGFs/IGFRs/IGFBPs system plays a crucial role in a variety of biological processes, including glucose and lipid metabolism (Beaune et al., 1992; Stanley et al., 2020), inflammation (Xu et al., 2013; Stanley et al., 2020), and growth and development (Stanley et al., 2020; Li et al., 2023). Consequently, the reduction in *IGFBP2* expression in muscle tissue could contribute to the development of fatty liver in geese by influencing the expression of genes involved in glucose and lipid metabolism, cell growth, and inflammation.

It is shown in this study that *IGFBP2* overexpression altered the expression of several glucose- and lipid metabolism-related genes (*LOC106030908*, *LOC106040417*, *PLPP4*) in primary goose pectoral muscle cells, upregulating *LOC106030908* and *LOC106040417*, while downregulating *PLPP4*. These and other downstream genes may play a role in mediating the effects of reduced *IGFBP2* expression in muscle during the development of fatty liver in geese. *LOC106030908* and *LOC106040417*could contribute to lipid accumulation, lipotoxicity, and insulin resistance, thereby accelerating the progression of the disease. Additionally, *IGFBP2* overexpression influenced the expression of inflammatory cytokines genes (*IL6, LOC106041919, LOC106038123, PTGS2, FGB*) in primary goose pectoral muscle cells. In specific, it upregulated *FGB* expression while suppressing *LOC106038123, PTGS2, IL6,* and *LOC106041919*. These findings further support the role of *IGFBP2* as a regulatory molecule linking metabolic and inflammatory pathways involved in the pathogenesis of fatty liver in geese. These downstream genes may mediate the inflammatory response triggered by reduced *IGFBP2* expression in muscle, exacerbating IRand promoting goose fatty liver formation.

This study measured the expression levels of genes significantly affected by *IGFBP2* overexpression, which are related to glucose-lipid metabolism and cytokines. The induced genes (*LOC106030908*, *FGB*, *LOC106040417*) and inhibited genes (*PLPP4*, *LOC106038123*, *PTGS2*, *IL6*, *LOC106041919*) were examined in both in vivo models (muscle tissue samples after 12 days of overfeeding) and in vitro models (primary goose muscle cell samples treated with glucose or insulin). The results indicated that overfeeding induced the expression of *PLPP4*, *IL6*, and *LOC106041919*, while it inhibited the expression of *LOC106030908*, *FGB*, and *LOC106040417*. Additionally, *IL6, LOC106041919, LOC106040417*, and *PTGS2* expression were induced by glucose, while *PLPP4* and *LOC106030908* expression were inhibited by glucose. Similarly, *PLPP4, LOC106041919*, and *LOC106040417* expression were induced by insulin, while LOC106030908 expression was inhibited by insulin. Based on the findings from both in vivo and in vitro models, *IGFBP2* appears to significantly influence the expression of genes involved in inflammation and glucose-lipid metabolism in muscle or muscle cells. This suggests that *IGFBP2* may play a role in promoting lipid accumulation and inflammation-induced IR in muscle, which could contribute to the development of fatty liver in geese. However, additional research is needed to confirm these results.

This study examines mRNA changes in isolated myocytes and does not account for inter-tissue communication. In vivo, *IGFBP2* regulation is shaped by endocrine factors such as thyroid hormone and by cell–cell interactions absent in culture. Future studies will include protein-level validation and liver–muscle co-culture or in vivo models to test the causal relationship between *IGFBP2* and insulin signaling.

CONCLUSION

This study demonstrates that excessive feeding significantly suppresses *IGFBP2* gene expression in the pectoral muscle during the development of fatty liver in geese. Elevated glucose and insulin levels, resulting in hyperglycemia and hyperinsulinemia, are associated with the inhibition of *IGFBP2* expression. These metabolic disturbances may contribute to the onset of IR and promote lipid accumulation in muscle tissue. In turn, this can influence the expression of genes involved in glucose and lipid metabolism, as well as the production of inflammatory cytokines. It is hypothesized that the downregulation of *IGFBP2* exacerbates fatty liver disease by further aggravating metabolic imbalances within muscle tissue. These findings highlight the critical role of *IGFBP2* in metabolic regulation and provide a foundation for further investigation into its role in poultry physiology and pathology.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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