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The role of insulin and glucose in goose primary hepatocyte triglyceride accumulation

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SUMMARY

In order to obtain some information on how fatty liver arises in geese, we investigated the role of insulin and glucose in triglyceride (TG) accumulation in goose primary hepatocytes. Goose primary hepatocytes were isolated and treated with insulin and glucose. Compared with the control group, 100 and 150 nmol l^{-1} insulin increased TG accumulation, acetyl-CoA carboxylase-α (ACCα) and fatty acid synthase (FAS) activity, and the mRNA levels of sterol regulatory element-binding protein-1 (*SREBP-1*), *FAS* and *ACC*α genes. Insulin at 200 nmol l^{-1} had an inhibiting effect on TG accumulation and the activity of ACC and FAS, but increased the gene expression of SREBP-1, FAS and ACCα. We also found that high glucose (30 mmol l^{-1}) increased the TG level, ACC and FAS activity, and the mRNA levels of SREBP-1 and FAS. However, there was no effect of high glucose on ACCα mRNA level. In addition, the interaction between insulin and glucose was observed to induce TG accumulation, ACC and FAS activity, and gene expression of SREBP-1, FAS and ACCα, and increase SREBP-1 nuclear protein level and binding of nuclear SREBP-1 and the SRE response element of the *ACC* gene. The result also indicated that the glucose-induced TG accumulation decreased after 96 h when the hepatocytes were cultured with 30 mmol l^{-1} glucose. In conclusion, insulin and glucose may affect hepatic lipogenesis by regulating lipogenic gene expression and lipogenic enzyme activity in goose hepatocytes, and SREBP-1 might play an important role in the synergetic activation of lipogenic genes. We propose that the utilization of accumulated TG in hepatocytes is the reason for the reversible phenomenon in goose hepatocellular steatosis.

Key words: glucose, goose primary hepatocytes, insulin, triglyceride accumulation.

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). In the case of pathological steatosis, the liver cells have degenerative lesions that are generally irreversible. During non-pathological hepatic steatosis, the functional integrity of the liver cells remains intact, and cellular hypertrophy is totally reversible (Babilé et al., 1996; Babilé et al., 1998; Bénard and Labie, 1998; Bénard et al., 1998). In order to find the mechanism of occurrence of fatty liver, some researchers have studied the hepatic steatosis of waterfowl, focusing on the synthesis and secretory pathway of hepatic lipoprotein, but there has been no report about the pathway of *de novo* synthesis of fatty acids.

De novo fatty acid synthesis in liver is regulated by insulin and glucose (Koo et al., 2001; Stoeckman and Towle, 2002). The lipogenic genes [including acetyl-CoA carboxylase-α (*ACC*α) and fatty acid synthase (*FAS*)] are activated by a combined effect of glucose and insulin in activating sterol regulatory element-binding protein-1 (SREBP-1) (Koo et al., 2001; Dentin et al., 2004). Two isoforms of SREBP have been identified in mammals: SREBP-1a and SREBP-1c. In chicken, however, only one form of SREBP-1 was observed, which was similar to the SREBP-1a in mammals (Zhang and Hillgartner, 2004). Previous studies have shown that SREBP-1c expression itself can only partly explain the glucose/insulin induction of lipogenic genes in primary cultured hepatocytes (Koo et al., 2001; Dentin et al., 2004; Stoeckman and Towle, 2002; Dentin et al., 2005). The transcriptional induction of *ACC*α and *FAS* genes requires both glucose and insulin (Dentin et al., 2005; Foufelle and Ferre, 2000). Thus, in order to understand the mechanism of hepatic steatosis, it is important to elucidate the role of insulin and glucose in triglyceride (TG) accumulation in waterfowl.

Sichuan white goose (*Anser cygnoides*) has a moderate capability to produce fatty liver, and it is suitable as a model system to understand the regulation of lipogenesis by insulin and glucose. We therefore isolated primary hepatocytes in Sichuan white geese as the experimental material. The present study was designed to investigate the regulation of lipogenesis by insulin and glucose in hepatocytes, which could be reflected by the effect of insulin and glucose on the accumulated lipids, ACC and FAS activity, the mRNA expression of SREBP-1, FAS and ACCα, and the binding of nuclear SREBP-1 and the SRE response element of the *ACC* gene.

MATERIALS AND METHODS

Primary hepatocyte isolation and culture

Hepatocytes were isolated from three 10 day old Sichuan white geese (*Anser cygnoides* Linnaeus 1758) from the Experimental Farm for Waterfowl Breeding at Sichuan Agricultural University by a modification of a previous method (Seglen, 1976). Freshly isolated hepatocytes were diluted to 1×10^6 cells ml^1. The culture medium was composed of DMEM (containing 4.5 g l^1 glucose; Gibco, Gaithersburg, MD, USA) with 100 IU l^1 insulin (Sigma, St Louis, MO, USA), 100 IU ml^1 penicillin (Sigma), 100 mg ml^1 streptomycin (Sigma), 2 mmol l^{-1} glutamine (Sigma) and 100 ml l^1 fetal bovine

serum (Clark, Tasmania, Australia). The hepatocytes were then plated in 60 mm culture dishes at 3×10^6 cells per dish for the preparation of total RNA and nuclear extracts, and plated in 24-well plates at 1×10^6 cells per well for measurement of TG level and ACC and FAS activity. Cultures were incubated at 40°C in a humidified atmosphere containing 5% CO_2, with medium renewed after 3 h, followed by serum-free medium, renewed after 24 h. After another 24 h, hepatocytes were separately treated with culture medium supplemented with 50 nmol l^{-1} insulin, 100 nmol l^{-1} insulin, 150 nmol l^{-1} insulin, 200 nmol l^{-1} insulin, 5 mmol l^{-1} glucose, 30 mmol l^{-1} glucose, 50 nmol l^{-1} insulin + 5 mmol l^{-1} glucose, and 50 nmol l^{-1} insulin + 30 mmol l^{-1} glucose for 48 h, while the control sample cells were cultured with serum-free medium for 48 h (serum-free medium was renewed every 24 h). In each case the experiments were repeated three times.

Isolation of total RNA and real-time RT-PCR

Total RNA was isolated from cultured cells using Trizol (Invitrogen, Carlsbad, CA, USA), and reverse transcribed using the PrimerScript™ RT system kit for real-time PCR (TaKaRa, Otsu, Japan) according to the manufacturer's instructions. The quantitative real-time PCR reaction mix contained the newly generated cDNA template, SYBR Premix Ex Taq™, sterile water, and primers of the target genes. Real-time PCR was obtained on the Cycler system (one cycle of 95°C for 10 s, 40 cycles of 95°C for 5 s, and 60°C for 40 s). An 80 cycle melt curve was performed, starting at 55°C and increasing by 0.5°C every 10 s, to determine primer specificity. Specific primers are listed in Table 1, designed according to the goose gene sequences: the *FAS* gene sequence was from GenBank (GenBank accession no. M60622); the *SREBP-1* and *ACC*α genes were sequenced in our lab (GenBank accession nos EU333990 and EF990142).

Amplicons corresponding to each target were examined by agarose gel electrophoresis to confirm the presence of a unique band of the expected size. Negative controls corresponding to PCR amplification with non-reverse transcribed RNA did not generate any signal. All samples were amplified in duplicate, with the same PCR mixture and in the same 96-well plate. The cycle threshold variation observed between duplicates was on average 0.12±0.1, demonstrating a high intra-assay reproducibility. Each sample was also replicated in another 96-well plate. The variation of Ct between two independent plates was 0.28±0.22, showing a fair interassay reproducibility as well. PCR products were then diluted 16-fold and were used to generate the calibration curve and the amplification rate (R) for each gene (*SREBP-1*, *ACC*α, *FAS* or *18S*). For each experimental sample, a normalized target gene level (Exp) corresponding to the target gene expression level relative to the *18S* (house keeping gene) expression level was determined by the $2^{-\Delta\Delta Ct}$ method as described previously (Livak and Sehmittgen, 2001):

$$\mathrm{Exp}_{\text{target gene}} = (1 + R_{\text{target gene}})^{\mathrm{Ct(target\ gene)}} / (1 + R_{18S})^{\mathrm{Ct}(18S)} . \quad (1)$$

For the target gene expression analyses, the normalized target gene expression level for each sample was compared with the positive control sample. Therefore, final results are expressed as N-fold differences in normalized target gene expression level between each treated and control sample.

Preparation of hepatocyte nuclear extracts

Nuclear extracts were prepared by a modified version of a procedure described previously (Azzout-Marniche et al., 2000). Briefly, cultured hepatocytes in 60 mm plates were scraped into PBS, combined, and centrifuged at 1000 ***g*** for 3 min. The cell pellet was resuspended in 2 ml of lysis buffer (10 mmol l^{-1} Tris-HCl, 0.3 mol l^{-1} sucrose, 10 mmol l^{-1} NaCl, 3 mmol l^{-1} $MgCl_2$, 0.5% Nonidet P40, 50 g ml^{-1} calpain inhibitor I, 1 mmol l^{-1} PMSF, 2 g ml^{-1} aprotinin and 10 g ml^{-1} leupeptin). After 15 min on ice, nuclei were pelleted by 10 min centrifugation (500 ***g***) at 4°C and washed once in the same buffer. The nuclear pellet was resuspended in 1 ml of hypertonic buffer (10 mmol l^{-1} Hepes, pH 7.4, 0.42 mol l^{-1} NaCl, 1.5 mmol l^{-1} $MgCl_2$, 2.5% glycerol, 1 mmol l^{-1} EDTA, 1 mmol l^{-1} EGTA, 1 mmol l^{-1} dithiothreitol, and the same protease inhibitors listed for the lysis buffer). After 30 min on ice, the nuclear extract was obtained by centrifugation at 100,000 ***g*** for 30 min at 4°C. Protein content was determined by spectrophotometry using a Bio-Rad protein assay reagent with bovine serum albumin as a standard.

SREBP-1 protein analysis by western blotting

Aliquots of nuclear proteins (40 µg for cell extracts) were separated by 10% SDS-PAGE and transferred to PVDF membrane. Membranes were then incubated with mouse anti-SREBP-1 monoclonal antibody (Lab Vision Corporation, Fremont, CA, USA). The primary antibody was used at a concentration of 4 g ml^{-1}. Signals were detected using an ECL western blot detection kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and goat anti-mouse horseradish peroxidase-conjugated IgG (Santa Cruz Biotechnology) as the secondary antibody. After analysis, the membranes were stripped with Re-Blot Plus solution (Chemicon International, Temecula, CA, USA) and blotted with α-tubulin antibody (TU-02, Santa Cruz Biotechnology) to normalize for protein level. The blot images were digitized with a luminescent image analyser (LAS-1000, Fuji Photo Film).

Electrophoretic mobility shift assay

EMSA was performed as described previously (Bobard et al., 2005). The double-stranded DNA fragment (5′-TCGCATCAC-ACCACCGCGG-3′) containing the SRE response element of the *ACC* gene was 5′-end labelled with γ-P^{32}ATP using T4 polynucleotide kinase. A typical reaction contained 100,000 c.p.m. (10–20 fmol) of ^{32}P-labelled oligonucleotide and 4 µg nuclear protein; 2 mg of poly(dIzdC) and 1.9 mg of poly(dAzdT) were used as non-specific competitors for the EMSAs. Following incubation at room temperature for 30 min, samples were subjected to electrophoresis on a 4.5% non-denaturing polyacrylamide gel and imaged by PhosphorImager analysis. SREBP-1 antibody was added to nuclear protein for 20 min at 4°C prior to the addition of the probe. For competitive binding, a 10-, 25- or 50-fold molar excess of unlabelled oligonucleotide was added together with radiolabelled probe prior to the incubation.

Table 1. PCR primers

Gene	Upstream primer (5′–3′)	Downstream primer (5′–3′)	Product size (bp)
SREBP-1	CGAGTACATCCGCTTCCTGC	TGAGGGACTTGCTCTTCTGC	92
FAS	TGGGAGTAACACTGATGGC	TCCAGGCTTGATACCACA	109
*ACC*α	TGCCTCCGAGAACCCTAA	AAGACCACTGCCACTCCA	163

SREBP-1, sterol regulatory element-binding protein-1; ACCα, acetyl CoA carboxylase-α; and FAS, fatty acid synthase.

Table 2. TG accumulation, and ACC and FAS activity in goose primary hepatocytes after treatment with insulin or glucose

Insulin (nmol l^{-1})	Glucose (mmol l^{-1})	TG accumulation (mmol l^{-1})	FAS activity*	ACC activity†
0	0	0.725±0.064^{c}	169.0±7.211^{d}	105.14±9.98^{c}
50	–	0.890±0.014^{c}	181.3±18.148^{d}	107.53±10.49^{c}
100	–	1.145±0.035^{b}	195.3±20.648^{c}	126.53±11.52^{b}
150	–	1.320±0.028^{a}	223.3±21.385^{b}	132.76±11.31^{b}
200	–	0.340±0.113^{d}	121.3±5.508^{e}	86.61±8.23^{d}
–	5	0.855±0.050^{c}	189.7±11.590^{d}	106.61±10.25^{c}
–	30	1.675±0.007^{a}	213.7±15.044^{b}	110.28±10.31^{c}
50	5	1.125±0.261^{b}	231.7±15.503^{b}	121.53±10.28^{b}
50	30	1.485±0.431^{a}	256.3±14.572^{a}	156.12±11.18^{a}

TG, triglyceride.

*Activity given as nmoles of substrate (NADPH) transformed to NADP per min per mg of cytosolic protein; †nmoles of substrate ($H[^{14}C]O_3^-$) fixed to malonyl CoA per min per g of cytosolic protein.

Different lowercase letters in the same array indicate a significant difference between treatments (P<0.05). After 24 h in serum-free medium, hepatocytes were incubated for 48 h either with no addition as a control or with 50 nmol l^{-1} insulin, 100 nmol l^{-1} insulin, 150 nmol l^{-1} insulin, 200 nmol l^{-1} insulin, 5 mmol l^{-1} glucose, 30 mmol l^{-1} glucose, 50 nmol l^{-1} insulin + 5 mmol l^{-1} glucose or 50 nmol l^{-1} insulin + 30 mmol l^{-1} glucose added. The standard culture medium contained 25 mmol l^{-1} glucose before additions.

Measurement of TG accumulation, and ACC and FAS activity

Samples of cultured cells for each treatment were shaken for 1 h using an ultrasonic processor, then washed three times with ice-cold phosphate-buffered saline and added to an isovolumic mixture of chloroform and methanol (2:1, v/v). The TG level was quantified by a colorimetric enzymatic method (Fossati and Prencipe, 1982) using a Triglyceride GPO-POD assay kit (Biosino, Beijing, China). The assay for FAS activity was performed according to Ingle et al. (Ingle et al., 1973), with some modifications. The FAS activity was calculated from the rate of transformation of NADPH to NADP in incubations containing substrate, cofactors and cell samples, by spectrophotometry at 340 nm. The concentration of the reagents was: 40 mmol l^{-1} potassium phosphate buffer plus EDTA (pH 6.8), 0.1 mmol l^{-1} malonyl CoA, 0.1 mmol l^{-1} acetyl-CoA, 0.3 mmol l^{-1} NADPH and 0.4 mmol l^{-1} dithiothreitol. ACC activity was measured as described previously (Majerus et al., 1968) with substantial modifications. The concentration of reagents was: 60 mmol l^{-1} Tris-HCl, pH 7.50, 2.1 mmol l^{-1} ATP, 5 mmol l^{-1} $MgCl_2$, 0.15 mmol l^{-1} acetyl-CoA, 1.2 mmol l^{-1} β-mercaptoethanol, 1 mg ml^{-1} fatty acid-free bovine serum albumin, 18 mmol l^{-1} $NaH[^{14}C]O_3$ (specific activity 0.5 mCi mmol l^{-1}) and 10 mmol l^{-1} sodium citrate. The assay was terminated by the addition of 1/6 volumes of 10% perchloric acid. An aliquot of the protein-free supernatant was dried in a counting vial under a hair dryer, then the residue was dissolved in a small volume of water and scintillation fluid was added. ACC activity is expressed as nmoles of substrate ($H[^{14}C]O_3^-$) fixed to malonyl CoA per min per g of cytosolic protein at 37°C. Protein concentration in the homogenate was determined by the Biuret method using bovine serum albumin as a standard, and activities are expressed as nmoles min^{-1} mg^{-1} of cytosolic protein. Analyses were performed in duplicate.

Statistical analysis

The data were subjected to ANOVA and the means were compared for significance by Tukey's test. Analysis of variance and t-test were performed using the SAS 6.12 package (SAS Institute, Cary, NC, USA). Results are presented as means ± s.d.

RESULTS

Effect of glucose and insulin on TG accumulation, and on ACC and FAS activity

As shown in Table 2, 100 and 150 nmol l^{-1} insulin could induce TG accumulation, but 200 mmol l^{-1} insulin had a marked inhibitory effect. High glucose (30 mmol l^{-1}) had a significant effect on TG accumulation (P<0.05). However, low glucose (5 mmol l^{-1}) had no effect. When cultured with 50 nmol l^{-1} insulin and low glucose (5 mmol l^{-1}) for 48 h, the TG accumulation in hepatocytes increased significantly (P<0.05), and when cultured with 50 nmol l^{-1} insulin and high glucose (30 mmol l^{-1}) the effect was even stronger (P<0.05).

Compared with the control group, Table 2 shows that at 100–200 nmol l^{-1} insulin increased FAS activity, with 150 nmol l^{-1} insulin having the greatest effect (P<0.05). Low (5 mmol l^{-1}) glucose had no effect on FAS activity, but high (30 mmol l^{-1}) glucose had a significant effect (P<0.05). Low glucose and 50 nmol l^{-1} insulin cultured together had an evident effect (P<0.05) on FAS activity, and high glucose and 50 nmol l^{-1} insulin together increased FAS activity too (P<0.05).

Table 2 shows that 100 nmol l^{-1} and 150 nmol l^{-1} insulin both increased (P<0.05) ACC activity, and 200 nmol l^{-1} insulin had an inhibitory effect (P<0.05). Low and high glucose both had no effect on ACC activity, but ACC activity could be up-regulated (P<0.05) by both low and high glucose together with 50 nmol l^{-1} insulin.

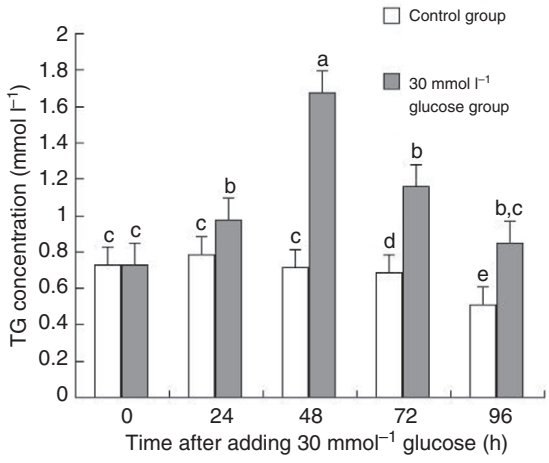

Fig. 1. Triglyceride (TG) accumulation in geese hepatocytes after 0–96 h in the presence of 30 mmol l^{-1} glucose or no glucose as control. Different lowercase letters indicate a significant difference between treatments (P<0.05). After 24 h in serum-free medium, hepatocytes were incubated in the presence of 30 mmol l^{-1} glucose or no glucose (control) for 24, 48, 72 and 96 h.

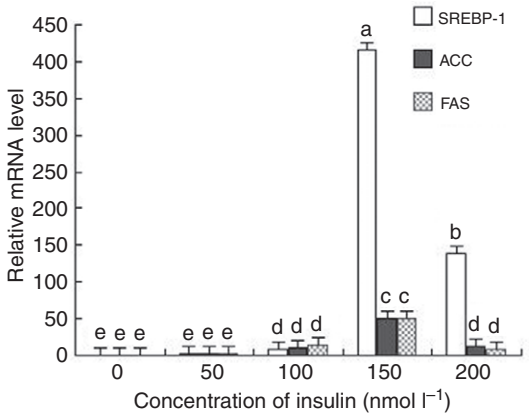

Fig. 2. Relative mRNA levels of SREBP-1, ACCα and FAS in goose primary hepatocytes treated with different concentrations of insulin. Different lowercase letters indicate a significant difference between treatments (P<0.05). After 24 h in serum-free medium, hepatocytes were incubated for 72 h either with no addition as a control or in the presence of 50, 100, 150 or 200 nmol l^{-1} insulin.

Fig. 1 shows that TG accumulation increased 24 h after 30 mmol l^{-1} glucose addition, reaching the highest level after 48 h. After 72 h, TG accumulation decreased, returning to the control level after 96 h. The change of TG accumulation in the control group was different from that of the treatment group, and the TG concentration decreased after 48 h in the control group.

Regulation of gene expression by insulin in goose primary hepatocytes

Fig. 2 shows that the regulation of *SREBP-1*, *FAS* and *ACC*α gene expression was similar. Insulin at 0–50 nmol l^{-1} had no evident effect on the mRNA level of the three genes. The gene expression was up-regulated by 100 nmol l^{-1} insulin and reached a maximum at 150 nmol l^{-1}, followed by a decrease at 200 nmol l^{-1}.

Glucose and insulin regulate the mRNA expression of SREBP-1 and lipogenic genes

Table 3 presents the synergetic effect of insulin and glucose on the mRNA expression of SREBP-1, FAS and ACCα by quantitative real-time PCR analysis. Low glucose did not have a significant effect on the mRNA expression level of SREBP-1 and FAS, but high glucose had significant effects on the amount of SREBP-1 and FAS mRNA. However, neither low nor high glucose had an evident effect on ACCα mRNA level. Insulin at 50 nmol l^{-1} and low glucose together had a significant stimulatory effect on expression of the three genes, and 50 nmol l^{-1} insulin and high glucose together had the greatest effect.

Synergetic effects of glucose and insulin on SREBP-1 translation

To determine whether glucose and insulin affect SREBP-1 translation, goose hepatocytes were exposed to glucose and insulin for 48 h. Fig. 3 shows the effects of glucose and insulin on SREBP-1 protein expression. After incubation with 50 nmol l^{-1} insulin or 5 mmol l^{-1} glucose there was no evident effect, but a significant increase in SREBP-1 protein level induced by 50 nmol l^{-1} insulin plus 5 mmol l^{-1} glucose was observed.

To confirm the role of SREBP-1 in mediating lipogenic gene expression induced by glucose and insulin in hepatocytes, an EMSA was performed to detect whether the DNA binding affinity of SREBP-1 increased after cells were cultured with glucose and insulin for 48 h. As shown in Fig. 4, binding of the nuclear SREBP-1 to the ACCα SRE sequence was induced by 50 nmol l^{-1} insulin plus 5 mmol l^{-1} glucose.

DISCUSSION

In mammals, several studies have shown that insulin may activate the transcription of SREBP-1, FAS and ACCα (Azzout-Marniche et al., 2000; Fleischmann and Iynedjian, 2000; Becard et al., 2001; Foretz et al., 1999a; Matsumoto et al., 2002), but an inconsistent report in chicken showed no effect of insulin on ACCα mRNA level (Zhang et al., 2003). In the present study, we found that insulin affected the mRNA expression level of SREBP-1, ACCα and FAS in goose primary hepatocytes. In particular, SREBP-1 was up-regulated 1000 times by 150 nmol l^{-1} insulin compared with controls, which was rarely found in other species. The great induction of SREBP-1 mRNA expression indicates that SREBP-1 may be the main pathway of lipogenesis induced by insulin in geese. In addition, TG accumulation, and ACC and FAS activity were stimulated by insulin. In mammals, insulin is the main hormone regulating the expression of SREBP-1, and it was found to not only induce the transcription of SREBP-1 but also stimulate the development of the mature form of SREBP-1, and so modulate hepatic lipogensis (Foretz et al., 1999a; Shimomura et al., 1999). Our results indicate that, consistent with the case in mammals, insulin may induce lipogenesis in goose liver by regulating the expression of SREBP-1 and lipogenic enzyme genes. Compared with 150 nmol l^{-1} insulin, 200 nmol l^{-1} insulin had an inhibitory effect on TG accumulation, ACC and FAS activity, and mRNA expression of SREBP-1, ACCα and FAS, which may be the result of the high concentration of insulin exceeding the tolerance of goose hepatocytes, leading to a decrease in insulin sensitivity, and an increase in resistance to insulin.

With respect to the influence of glucose on the expression of SREBP-1 and lipogenic enzymes, previous studies have shown inconsistent findings. Some investigators have reported that glucose activated SREBP-1 expression in a mouse hepatocyte cell line (Hasty et al., 2000), whereas others found that in primary rat hepatocytes

Table 3. Relative mRNA levels in goose primary hepatocytes treated with insulin and glucose

Insulin (nmol l^{-1})	Glucose (mmol l^{-1})	SREBP-1	ACCα	FAS
0	0	1±0.004^{c}	1±0.009^{c}	1±0.008^{d}
0	5	2.97±0.106^{c}	1.74±0.014^{c}	1.16±0.027^{d}
0	30	7.67±0.071^{b}	0.41±0.004^{c}	59.10±5.122^{b}
50	5	12.51±1.011^{b}	3.86±0.116^{b}	4.08±0.071^{c}
50	30	1105.13±10.211^{a}	22.24±2.223^{a}	97.68±7.771^{a}

Different lowercase letters in the same row indicate a significant difference between treatments (P<0.05). After 24 h in serum-free medium, hepatocytes were incubated for 48 h with either no addition as a control or 5 mmol l^{-1} glucose, 30 mmol l^{-1} glucose, 50 nmol l^{-1} insulin + 5 mmol l^{-1} glucose, 50 nmol l^{-1} insulin + 30 mmol l^{-1} glucose. The standard culture medium contained 25 mmol l^{-1} glucose before additions.

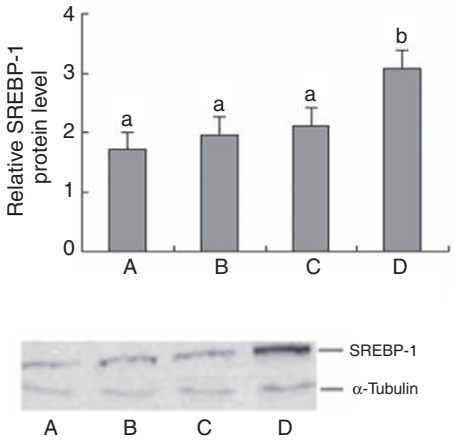

Fig. 3. Immunoblot analysis of nuclear SREBP-1 in goose hepatocyte nuclear extracts treated with insulin and glucose. (A) control, (B) 50 nmol l^{-1} insulin, (C) 5 mmol l^{-1} glucose, (D) 50 nmol l^{-1} insulin + 5 mmol l^{-1} glucose. After 24 h in serum-free medium, hepatocytes were incubated for 48 h with insulin and glucose. The standard culture medium contained 25 mmol l^{-1} glucose before additions. Each blot is representative of two independent experiments. Different lowercase letters indicate differences between treatments (P<0.05).

and rat livers glucose potentiated the effect of insulin on SREBP-1c expression but had no effect in the absence of insulin (Shimomura et al., 1999; Foretz et al., 1999b). Recently, Matsuzaka and colleagues demonstrated that at least part of the controversy probably results from species differences, and glucose can activate expression of SREBP-1c in mouse liver independent of insulin, whereas the activation of SREBP-1c expression by glucose in rat liver is very limited in the absence of insulin (Matsuzaka et al., 2004). The current study shows that glucose is the main inducer of hepatic lipogenesis. The increase in TG accumulation and FAS activity induced by glucose was more evident than that by insulin. We compared TG accumulation, FAS activity, and mRNA expression of SREBP-1, ACCα and FAS of goose primary hepatocytes cultured in either low or high glucose-containing medium. It is likely that culture in medium containing low glucose might be comparable with normal feeding conditions. In contrast, when cells were cultured in the presence of high glucose, TG accumulation, and SREBP-1c and FAS levels increased significantly, which might be equivalent to an overfeeding situation

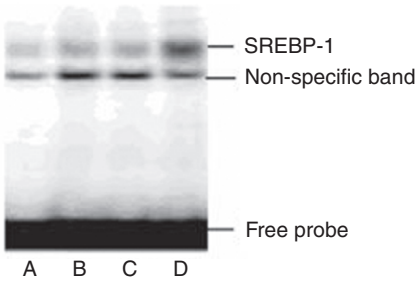

Fig. 4. EMSA performed with goose hepatocyte nuclear extracts treated with insulin and glucose. (A) control, (B) 50 nmol l^{-1} insulin, (C) 5 mmol l^{-1} glucose, (D) 50 nmol l^{-1} insulin + 5 mmol l^{-1} glucose. After 24 h in serum-free medium, hepatocytes were incubated for 48 h with insulin and glucose. The standard culture medium contained 25 mmol l^{-1} glucose before additions. Each blot is representative of two independent experiments.

(Kim and Freake, 1996; Zhang and Hillgartner, 2004). The TG accumulation returned to the control level 96 h after 30 mmol l^{-1} glucose addition. This is very similar to the reversible phenomenon. When the energy level is insufficient, TG is hydrolysed to supply the energy required by the liver. So the utilization of accumulated TG in hepatocytes is the reason for the reversible phenomenon in goose hepatocellular steatosis. In agreement with this, the current study indicated that glucose in excess is responsible for inducing fatty liver in geese.

Glucose and insulin display a marked synergism in lipogenesis in mammals (Koo et al., 2001; Vaulont et al., 2000), and our results in goose primary hepatocytes are consistent with these previous findings; insulin could increase glucose uptake and utilization. In the presence of insulin, low glucose increased TG accumulation, ACC and FAS activity, expression of *SREBP-1*, *ACC*α and *FAS* genes, and the protein level of nuclear SREBP-1. In the presence of insulin, the effect of high glucose reached a maximum. The EMSA results indicated that SREBP-1 might play a role in the synergetic activation of lipogenic genes induced by glucose and insulin. One potential explanation for the maximum effect requiring both insulin and high glucose could be linked to the fact that the glucose carbon atoms are orientated towards lipid synthesis only if glucose is particularly abundant. This is consistent with our previous study that the plasma concentrations of glucose and insulin were both higher in Landes geese, which have a more fatty liver than normal geese (Han et al., 2008). It is indicated that the metabolism of insulin and glucose are closely related to lipogenesis, and upsetting their metabolic balance may affect the regulation of *SREBP-1*, *ACC*α and *FAS* gene expression, and result in the accumulation of lipids in hepatocytes and so cause hepatocellular steatosis.

In conclusion, we found that both insulin and glucose could induce the mRNA expression of SREBP-1 and several lipogenic genes, and stimulate ACC and FAS activity, which may relate to the elevated accumulation of TG in hepatocytes. In addition, glucose may affect hepatocellular lipogenesis through the interaction with insulin. Our results indicate that the activation of ACCα and other downstream SREBP-1 targets by insulin and glucose in goose primary hepatocytes is likely to be secondary to the stimulation by SREBP-1.

LIST OF ABBREVIATIONS

ACCα	acetyl-CoA carboxylase-α.
FAS	fatty acid synthase
SREBP-1	sterol regulatory element-binding protein-1
TG	triglyceride

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