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Endogenous LPS alters liver GH/IGF system gene expression and plasma lipoprotein lipase in goats

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Summary

Endotoxin lipopolysaccharide (LPS) affects the ruminant health and animal performance. The main purposes of this study were to investigate the potential effects of GH/IGF system and lipoprotein lipase (LPL) concentration on resistance the circulating LPS concentration increased in liver with high concentrate diet treatment. Non-lactating goats were randomly allocated to two groups: a high-concentrate diet (HCD) or a low-concentrate diet (LCD) in cross over design and the blood collection at different time points after feeding at the end of the experiment. The average rumen pH was significantly reduced (P<0.05), but the duration with pH was not more than 120 min in the HCD group. The plasma LPL concentration was significantly raised (P<0.05).However, from 2 h onwards, LPS concentration was significantly reduced (P<0.01) in the HCD group compared with LCD group. In addition, the plasma IGF1 concentration and the hepatic insulin-like growth factor-1 receptor (IGF1R) mRNA expression were markedly reduced (P<0.05). However, growth hormone (GH) secretion at 15, 30, and 45 min after feeding and growth hormone receptor (GHR) mRNA expression in the liver was significantly increased (P<0.05) in HCD group. The correlation analysis showed that the plasma LPL concentration was positively correlated with hepatic GHR mRNA expression (P<0.05). Conversely, the plasma LPS concentration was negatively correlated with LPL concentration (P<0.05). These findings reveal that alterations in GH/IGF system function in response to a high-concentrate diet are accompanied by corresponding changes in systemic LPL in non-lactating goats' liver in presence of endogenous LPS stress.

Key words: endotoxin lipopolysaccharide; GH/IGF system; lipoprotein lipase

1. Introduction

Current feeding practices for ruminants use highly fermentable diets to maximize energy intake to change the environment in the rumen and decrease the ruminal pH or cause ruminal acidosis (Krause and Oetzel, 2006), with a loss of animal performance (Stone, 2004). One consequence of such feeding is to increase the rate of endotoxin lipopolysaccharide (LPS) (Emmanuel *et al.*, 2008; Khafipour *et al.*, 2009). High concentrate diets can cause a 20-fold increase in LPS release within rumen (Andersen *et al.*, 1994a). The phenomenon of high concentrate diet induced LPS production was defined as endogenous LPS and result in the stress (LPS stress), which caused rumen papillae damage (Steele *et al.*, 2009). Moreover, papillae damage allows entry of LPS into the blood and leads to generalized effects (Andersen *et al.*, 1994a). However, the blood LPS is cleared from the portal circulation by the liver (Andersen *et al.*, 1994b; Harris *et al.*, 2002), a process that involves macrophages (Kupffer cells) or neutralization by lipoproteins (Kasravi *et al.*, 2003a).

Thus, the liver plays a central role in clearing toxins trans-located into blood from inflammatory sites (Waldron *et al.*, 2003).

In ruminants alterations in the growth hormone (GH)-insulin-like growth factor (IGF) system occur in response to nutritional stress (Lee *et al.*, 1997; McGuire *et al.*, 1992). Alteration of dietary nutrition could mark effects on plasma GH and insulin like growth factor 1 (IGF1) concentration and mRNA abundance within GH/IGF system in sheep (Hua *et al.*, 1995; O'Sullivan *et al.*, 2002), heifers (Nosbush *et al.*, 1996), beef steers (Thorp *et al.*, 2000), bulls (Renaville *et al.*, 2000) and calves (Smith *et al.*, 2002). Importantly, activated GH/IGF activity are associated with lipoprotein enhancement and considered the main factors affecting IGF1 status in mammals with the nutrition alteration (Goldstein and Phillips, 1991; Lee *et al.*, 1997). Nutritional deficiency, induced by food deprivation or restriction, suppresses the hepatic gene expression of GHR (Dauncey *et al.*, 1994; Pell *et al.*, 1993; Sohlstrom *et al.*, 1998; Straus and Takemoto, 1990; Weller *et al.*, 1994). Previous results showed to impair GH/IGF activity could decrease the plasma high density lipoprotein and low density lipoprotein concentration (Sherlock and Toogood, 2007). Additionally, the lipoprotein lipase (LPL) enhances the affinity with lipoprotein to binding LPS and transfers into hepatocytes detoxification (Kasravi *et al.*, 2003b). However, little is known about how mediates or interaction the LPS detoxification in the liver with the nutrition alteration in ruminant.

The objective of this study was to test the hypothesis that changing the level of feed concentrates in the diet leads to alterations in the plasma LPS concentration that are accompanied by corresponding changes in plasma LPL and hepatic mRNA expression within GH/IGF system as a way to resist endogenous LPS stress, in order to resume production performance.

2. Materials and methods

2.1. Experimental design and goat management

Twelve 2-year old non-pregnant, non-lactating female Saanen goats were housed and treated in accordance with the guidelines established by the People’s Republic of China regarding animal welfare. All procedures were pre-approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University. Before the experiments, the goats were dewormed with oral Albendazole (15 mg/kg body weight) and subcutaneous Ivermectin (0.2 mg/kg body weight), and were acclimated to individual pens (120×100 cm). All goats were installed with rumen fistulae and kept under uniform management condition for adaptation to the new environment during one week. The goats were randomly allocated to two groups: a high-concentrate diet (HCD, n=12) group and a low-concentrate diet (LCD, n=12) group in a cross over design. Diets were formulated to meet or exceed the minimum nutrient requirements as recommended by NRC (2001) using the Cornell-Penn-Miner System (Table 1). The goats were fed twice daily at 0800 and 2000. Water was freeing available. The each feeding period lasted 42 d.

2.2. Sample collection and analysis

2.2.1. Sample collection

On day-42 of each diet period, 16 blood samples collected by jugular vein puncture into heparinised vacutainers at 15 min intervals for 4 h from 0800 after 42d on each period for measuring growth hormone. The samples were immediately placed on ice and within 20 min of collection, centrifuged at 3000×g at 4°C for 10 min and stored at -20°C until analysis. The same time sample of rumen fluid was collected by filtration through a cotton cloth at 15 min intervals for 4 h from 0800 after 42d on each period. After measured the pH value for each sampling point, the sample was stored at -20°C until analysis. The liver was collected by biopsy in the first period. At the end of the experiment, the goats were slaughtered by captive bolt followed by exsanguinations in the second period feeding. Liver tissue was collected washed twice with cold physiological saline (0.9% NaCl solution) to remove blood and other possible contaminants, and then transferred into liquid N and stored at -80 °C until analysis. The slaughter and sampling procedures complied with the "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398 set by the Ministry of Science and Technology, China and the "Regulation regarding the Management and Treatment of Experimental Animals" (2008) No. 45 set by the Jiangsu Provincial People's Government.

2.2.2. Measured plasma IGF1, LPL, LPS and GH concentration

The IGFI, LPL and LPS concentration of plasma (sampling point at 0h, 2h, and 4h) was measured. The IGF1 concentration measured using a competitive ^{125}I-RIA kit with an anti-IGF1 raised in rabbits and an anti-rabbit precipitant (goat). The kit purchased from Beijing North Institute of Biological Technology (Beijing, China). Brief, IGF1 separated from binding proteins by acid/ethanol (12.5% of 2 mol/L HCl and 87.5% ethanol) precipitation and each sample was analyzed in duplicate. Diluted plasma concentrations paralleled the standard curve indicating that the plasma IGF1 and IGF1 of standards were immunologically similar. The intra-assay (precision) and inter-assay CV (reproducibility) were 2 and 4.5%, respectively.

Plasma LPL concentration was determined using the Total Lipoprotein Lipase Detection Kit, purchased from Jiancheng Biotechnology Institution (Nanjing, China). Plasma LPS concentration was measured using the Goat-LPS Elisa Assay Kit according to the manufacturer's instructions after diluting the samples 1:3 with pyrogen-free water and. The kit was purchased from Shanghai Lengton Bioscience Co. (Shanghai, China).

Plasma LPS results was used a 96-well micro-plate with absorbance read at 450 nm on a micro-plate reader (RT-6000, RayTo).

Measured the GH concentration using 16 blood samples through a competitive ^{125}I-RIA kit with an anti-GH raised in rabbits and an anti-rabbit precipitant (donkey), purchased from Beijing North Institute of Biological Technology (Beijing, China). Each sample was analyzed in duplicate. Diluted plasma concentrations paralleled the standard curve indicating that the plasma GH and GH of standards were immunologically similar. The parallelism was described previously (Hashizume *et al.*, 2005). The intra-assay (precision) and inter-assay CV (reproducibility) were 4.8 and 6.1%, respectively.

2.2.3. Assessment of GH receptor and IGF1 receptor mRNA

The methods for total RNA extraction and RNase protection were as described elsewhere (Katsumata *et al.*, 2000). 0.5 g liver tissue was used TRIZOL (Invitrogen, Beijing, China) to isolate total RNA which quantified by measuring absorbance at 260 nm in a NanoDrop ND-1000 Spectrophotometer (Desjardins and Conklin, 2011). cDNA was generated from 2 μg of total RNA from each of the tester populations, and was converted by M-MLV reverse transcriptase (Promega, USA) as indicated by the manufacturer (protocol: heated to 95°C for 2 min, kept for 5 min at 70°C and then chilled on ice. cDNA was generated for 1 h at 37°C).

Real-time PCR was performed using a SYBR Green PCR Master Mix (Roche, Germany) in a Bio-Rad MyiQ™ Detection System (Applied Biosystems), according to the manufacturer's instructions. The abundances of GHR and IGF1R mRNA were determined. The relative amount of mRNA for each target gene was determined from the ratio against the mRNA of β-actin. The thermal cycling conditions were 2 min at 50°C, and 10 min at 95°C, followed by 40 repeats at 95°C for 20 s, 60°C for 45 s, and 72°C for 30 s min in a Bio-Rad MyiQ™ Detection System (Applied Biosystems, USA). According to the comparative threshold cycle (Ct) method, the amount of target mRNA normalized to β-actin and relative to an internal control was calculated by $2^{-\Delta\Delta Ct}$. The GH receptor (GHR) primers (Invitrogen, Shanghai, China) was forward (5'-TCCAG-CCTCTGTTTCA-3') and reversed (5'-CCACTGCCAAGGTCAA-3'), IGF1 receptor (IGF1R) primers was forward (5'-GCTCACCCAGGGAACTACAC-3') and reversed (5'-CCACTATCAACAGAACCGCAAT-3'), and β-actin primer was forward (5'-CGGGATCCATCCTGCGTCTGGACCTG-3') and reversed (5'-GGAAT-TCGGAAGGAAGGCTGGAAGAG-3').

2.3. Statistical analysis

Data are expressed as means ± SEM. Data for parameters of the IGF system in blood and liver tissue and differences in rumen pH content were analyzed by ANOVA. Differences with $P<0.05$ were considered to be significant.

3. Results

3.1. Rumen pH

The rumen pH was lower (6.47 and 6.05) between the LCD group and HCD group ($P<0.05$, Table 2). There was no difference in the duration of rumen pH below 6.0, but has an obvious increased at the time pH below 5.8 ($P<0.05$). Overall， for both diet the duration with ruminal pH below 5.8 was less than the 180 min considered threshold on the post-feeding for subacute ruminal acidosis (SARA).

3.2. Plasma LPS concentration

Plasma LPS concentration was lower ($P<0.05$) in the HCD than that in the LCD group (Fig. 1). No change in LPS concentration at 0 h was observed following treatment with the different diets ($P>0.05$) (Fig. 1). In the LCD group, plasma LPS concentration decreased from 74.4±5.7 EU/L at 0 h to 59.9±8.1 EU/L ($P<0.01$) at 2 h after feeding and consecutively remained lower ($P<0.01$). In the HCD group, plasma LPS concentrations decreased from 2 h onwards, and were lower ($P<0.01$) than those in the LCD group.

3.3. Plasma LPL, IGF1 and GH concentration

Marked differences in the plasma indices of LPL and IGF1 were observed between the two feeding groups (Table 2). The HCD treatment induced an increase in LPL concentration ($P<0.05$) compared to the LCD group, but the plasma IGF1 concentration was lower ($P<0.05$, Table 2). Plasma GH secretion responses to a HCD supplementation in non-lactation Saanen goats are shown in Fig. 2. The mean plasma GH concentrations in the LCD control goats varied within the range of 2.0 to 4.1ng/ml, and the LCD supplementation did not alter basal GH concentrations significantly during the post-feeding 4h. The HCD supplementation significantly stimulated GH release ($P<0.05$). The average GH levels in goats began to rise just at the post-feeding, and were significantly increased at 15 min (8.9±1.1 ng/ml), 30 min (7.4±0.9 ng/ml) and 45 min (5.1±0.6 ng/ml) after the feeding in the HCD group compared with the respective sampling time in the LCD group ($P<0.05$).

3.4. The GH/IGF system gene mRNA expressions in liver

The abundances of hepatic GHR mRNA expression was achieved 2.3-fold, higher in the HCD group than that in the LCD group ($P<0.05$, Fig. 3A). Incontrast, hepatic IGF1R mRNA expression was markedly reduced in the HCD group in comparison with the LCD group ($P<0.05$, Fig. 3B).

3.5. The relationship with the composition of GH/IGF system in liver, plasma LPL and plasma LPS

Plasma LPL concentration was positively correlated with GHR mRNA expression in the liver (R^2=0.8706, P=0.036, Fig. 4A). Conversely, there was a negative correlation between the plasma LPS and GHR mRNA expression in the liver across treatment in (R^2=0.892, P<0.000, Fig. 4B). There was a negative correlated at 0h (R^2=0.4956, P=0.01; Fig. 4C), 2 h (R^2=0.8517, P<0.000, Fig. 4D) and 4 h (R^2=0.7595, P<0.05, Fig. 4E).

4. Discussion

Although rumen pH varies considerably within a day, ruminant possess a highly developed system to maintain the pH within a physiological range. Nonetheless, if acid production from fermentation exceeds the buffering capacity, ruminal pH compensation fails and the pH may drop markedly. Previous studies showed that feeding practices in ruminants that use highly fermentable diets, or high concentrate diets, can exhibit decreased ruminal pH (Krause and Oetzel, 2006; Stone, 2004). Our study showed that the pH was decreased in the HCD group but still exceeded 5.8-6.0 (Table 2), above the pour of SARA (Gozho *et al.*, 2007). Previous studies have reported that intensive feeding of ruminants increased the rate of endotoxin LPS (Emmanuel *et al.*, 2008; Khafipour *et al.*, 2009), but it was clearance or inactivated from the portal circulation by the liver (Andersen *et al.*, 1994b). The results by Harris et al. showed that endotoxin was transferred to blood and cleared by liver macrophages or neutralized by lipoproteins (Harris *et al.*, 2002). According the LPS result (Fig. 1) showed that LPS was decreased could be attributed to clearance or partially clearance in the liver. However, the exact mechanism about the LPS was clearance should be further investigation in the future.

The IGF1 and IGF1R have a central role in growth regulation and are highly sensitive to nutritional status (Takenaka *et al.*, 1996). Studies have shown that fatty acid supplementation as endogenous ligands for peroxisome proliferator activated receptor (PPAR) also regulate the secretion and transcription of IGF system components (Brown *et al.*, 2003). In this trial, the HCD supplementation decreased IGF1R mRNA expression in the liver of non-lactating Saanen goats (Fig. 3B). Furthermore, the plasma IGF1 concentration decreased in the HCD group (Table 2). The current data confirm the findings of Richards et al. (Richards *et al.*, 1991) that nutritional alteration of cycling anestrus animals is associated with decreased circulating concentrations of IGF1.

GH secretory patterns in ruminants are different from that in human. In human the majority of GH is secreted during the night, within a few hours (Casanueva, 1992). Incontrast, GH secretion appears asynchronous and episodic, and irregular episodic GH pulses occur in ruminant (Hashizume *et al.*, 2005). In the present studies, the high concentrate supplementation significantly stimulated the release of GH after feeding at 15, 30 and 45 min (Fig. 2). GHR has a major somatogenic role could be more responsive to endogenous GH secretion in liver associated nutrition alteration (Katsumata *et al.*, 2000). Additional, Katsumata et al. (Katsumata *et al.*, 2000) explained that up-regulation of GHR mRNA expression in response to high concentrate diet intake can be considered as an adaptation the expense of the body growth and development. However, the association between nutrition and stress in endogenous GH secretion is not known in ruminants. Recent vitro studies showed GH could enhance the transcript levels of GHR mRNA in primary hepatocytes (Fang *et al.*, 2012). This raises the hypothesis that the nutritionally induced up-regulation of GHR expression, together with changes in other hormone levels (Fang *et al.*, 2012), may alter metabolism influx in liver to resist the endogenous LPS stress. Especially, LPS could be internalized through the hepatic endosomal pathway via lipoprotein receptors (Harris *et al.*, 2002). In dairy cows, stimulation of GHR abundance by GH to alter the situation of energy deficit leads to reduced expression of the liver GHR gene transcription (Rhoads *et al.*, 2007), and alterations in plasma lipide levels (Birzniece *et al.*, 2009). The regulation of non-lactating goat GHR mRNA by the high-concentrate diet in the present study was accompanied by variations in plasma LPL, especially increased plasma LPL content (Table 2). The LPL serves as a bridge between the cell surface and lipoproteins (Beisiegel *et al.*, 1991; Wong *et al.*, 1994), and bound LPS could rapidly attenuate the hepatocellular response to cytokines in a selective manner, mediated by lipoprotein receptors (Kasravi *et al.*, 2003a). It is interesting that there was a significant negative correlation between plasma LPL and LPS concentration in difference sampling times (Fig. 4C, Fig. 4D and Fig. 4E). According to the previous result, which showed C-terminal domains of LPL have a higher affinity for large triglyceride-rich lipoproteins compared with cholesterol-rich lipoprotein (Lookene *et al.*, 2000). Moreover, increasing the triglyceride-rich lipoproteins could depress the toxicity of LPS, thereby increased plasma LPL concentration has a potential role in depressing the damage from the endogenous LPS in the liver (Kasravi *et al.*, 2003b). As such, we postulate that the changes in hepatic GHR mRNA expression may contribute to the regulation of hepatic LPL activity, which enhances the binding of triglyceride-rich lipoproteins, transferring LPS to high-density lipoprotein and promoting circulating LPS inactivation (Kitchens *et al.*, 2001). Therefore, we can presume the alteration of GH/IGF system and LPL interaction resulting in the endogenous LPS degradation or detoxification in the high-concentrate diet treatment. To our knowledge, this is the first report demonstrating the potential connection between the GH/IGF system in the non-lactating goat and the resistance of the liver to chronic stress. Therefore, further studies are required to confirm this interaction.

Regardless of the mechanism, these data further highlight an important inter-relationship between hepatic

GHR and IGF1R mRNA expression with the host response to endogenous LPS stress. These changes were accompanied by variations in the plasma LPL concentration and the GH secretion. Therefore, the GH/IGF system and LPL activity may play an important role in the liver to resist or clear endogenous LPS.

Author contributions

Conceived and designed the experiments: YZ. Performed the experiments: ZX PY KZ. Analyzed the data: ZX. Contributed reagents/materials/analysis tools: YZ YN SZ XS. Wrote the paper: ZX. Read and approved final manuscript: ZX PY XJ YZ YN SZ XS.

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Table 1 Ingredient and nutrient composition of high-concentrate diet (HCD) and low-concentrate diet (LCD).

Item	HCD	LCD
Ingredients		
Hay	32.00	48.00
Purple medic	8.00	12.00
Corn	43.75	28.78
Soybean meal	12.68	8.45
Limestone	1.15	0.77
Calcium Hydrogen Phosphate	1.65	1.10
Salt	0.60	0.40
Premix[1]	0.75	0.50
Nutrients composition		
Net energy (MJ/Kg)	5.89	5.40
Crude protein (%)	13.75	12.24
Neutral detergent fiber (%)	27.69	36.55
Acid detergent fiber (%)	17.54	24.04
Calcium (%)	1.05	0.87
Phosphorus (%)	0.51	0.40

[1]Provided per kg of premix: Vitamin A, 6 000U; Vitamin D2, 500U; Vitamin E, 80mg; Cu, 6.25mg; Fe, 62.5mg; Zn, 62.5mg; Mn, 50mg; I, 0.125mg; Co, 0.125mg; Mo, 0.125mg.

Table 2 Effects of two experimental diets on ruminal pH, plasma LPL and IGF1 concentration in non-lactating Saanen goats.

Item	**LCD**	**HCD**	**SEM**	***P*-value**			
				group	diets	times	group×times×diets
Average ruminal pH	6.47[a]	6.05[b]	0.063	0.015	0.021	0.067	0.048
Total time<pH 6.0 (min/4h)	87.0	118.9	19.38	0. 27	0.42	0.94	0.76
Time at<pH 5.8 (min/4h)	27.3 [a]	53.4 [b]	9.09	0.035	0.046	0.87	0.57
Plasma LPL (U/mg●prot)	2.03[a]	3.903[b]	0.067	<0.000	<0.000	<0.000	<0.000
Plasma IGF1 (ng/ml)	7.564[a]	4.900[b]	0.127	0.007	0.003	0.013	0.009

[a, b] Means within the same row followed by different superscript letters differ significantly ($P < 0.05$). Values are mean ± SEM, n=12/group.

Figure legends

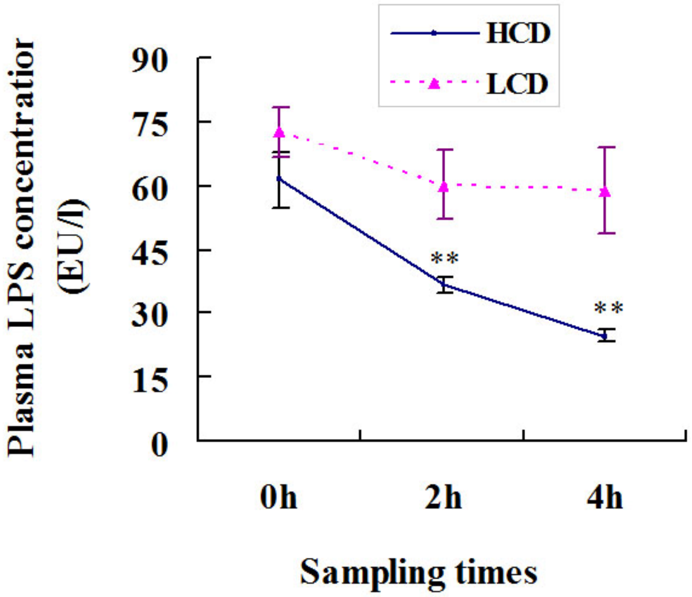
Figure 1 LPS concentrations in non-lactating Saanen goats fed a high concentrate diet (HCD) or low concentrate diet (LCD). Values are means ± SEM, n=12/group. ** indicates significant difference between HCD and LCD, $P<0.01$.

Figure 2 Plasma GH concentrations in non-lactating Saanen goats fed a high-concentrate diet (HCD) or low-concentrate diet (LCD). Each value represents the mean ± SEM for twelve animals. *$P<0.05$, **$P<0.01$ compared with the corresponding values for controls.

Figure 3 Liver GHR and IGF1R mRNA expression in non-lactating Saanen goats fed a high-concentrate diet (HCD) or low-concentrate diet (LCD). RNA molecules extracted from liver tissue were reverse transcribed to cDNA and analyzed by real-time PCR. Data expressed as arbitrary units relative to β-actin mRNA. A, GHR mRNA expression; B, IGF1R mRNA expression. Values are mean ± SEM, n=12/group. *indicates significant difference between HCD and LCD, $P<0.05$.

Figure 4 Pearson's correlation between plasma LPL concentration and hepatic GHR mRNA expression or plasma LPS concentration in non-lactating Saanen goats fed high concentrate diet. A, plasma LPL concentration vs. hepatic GHR mRNA expression; B, plasma LPS concentration vs. hepatic GHR mRNA expression; C, plasma LPS concentration vs. plasma LPL concentration (0 h); D, plasma LPS concentration vs. plasma LPL concentration (2 h); E, plasma LPS concentration vs. plasma LPL concentration (4 h). Values are mean ± SEM, n=12/group. The results indicate a significant correlation through the P value at the 0.05 or 0.01 levels.

Fig. 1



Note: ** means *P*<0.01

Fig. 2

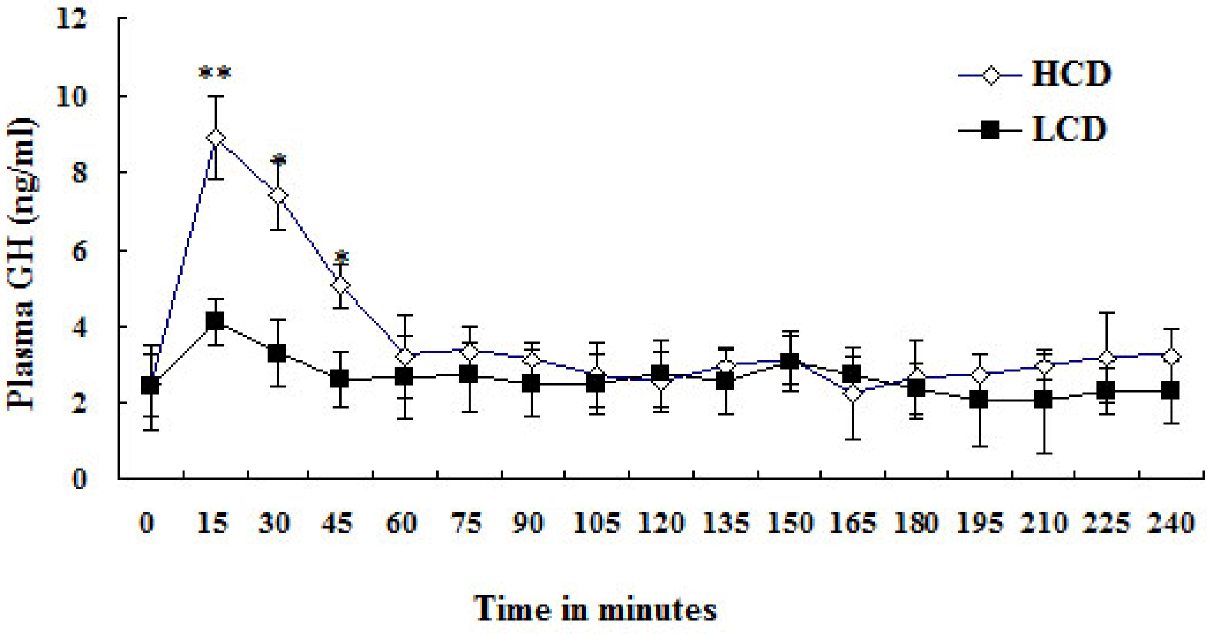
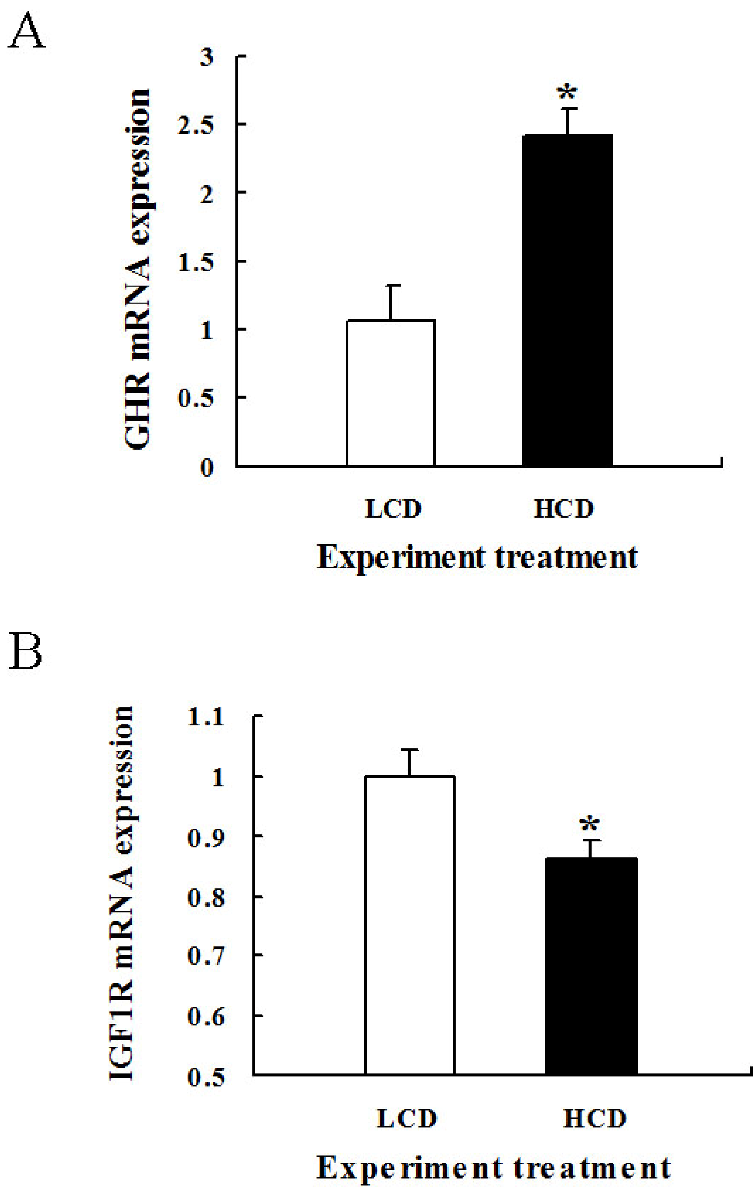
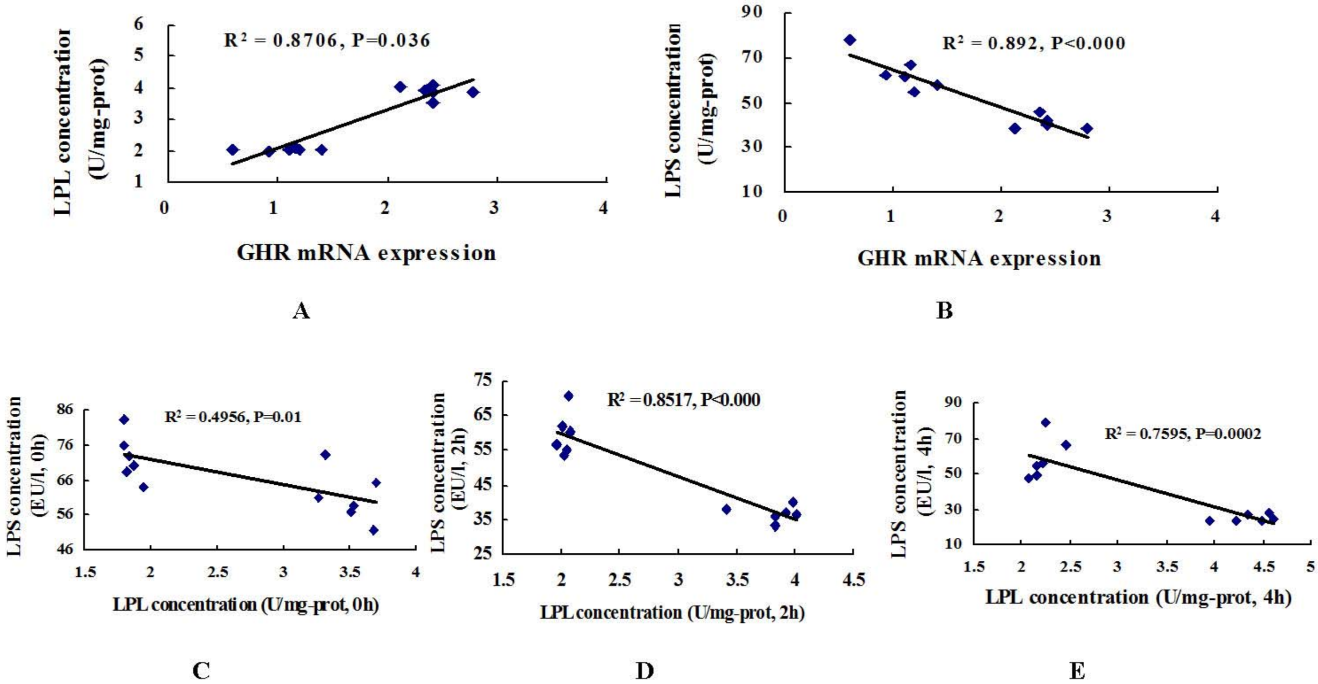


Fig. 3



A
GHR mRNA expression
3
2.5
2
1.5
1
0.5
0
*
LCD
HCD
Experiment treatment
B
IGF1R mRNA expression
1.1
1
0.9
0.8
0.7
0.6
0.5
*
LCD
HCD
Experiment treatment

Fig. 4



R² = 0.8706, P=0.036
LPL concentration (U/mg-prot)
GHR mRNA expression
A
R² = 0.892, P<0.000
LPS concentration (U/mg-prot)
GHR mRNA expression
B
R² = 0.4956, P=0.01
LPS concentration (EU/l, 0h)
LPL concentration (U/mg-prot, 0h)
C
R² = 0.8517, P<0.000
LPS concentration (EU/l, 2h)
LPL concentration (U/mg-prot, 2h)
D
R² = 0.7595, P=0.0002
LPS concentration (EU/l, 4h)
LPL concentration (U/mg-prot, 4h)
E