# 读书报告

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# The variation of serum glucose, hepatic glycogen content and expression of glucose metabolism-related genes in hybrid grouper (female *Epinephelus fuscoguttatus* × male *Epinephelus lanceolatus*) in response to intraperitoneal insulin infusion

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## Abstract

The present study was conducted to investigate the effects of insulin on serum glucose, hepatic glycogen content and expression of glucose metabolism-related genes in hybrid grouper. Triplicate groups of five fish were sampled at the 0, 1, 3, 6, 12, 24 or 48-h time point post-bovine insulin (5 μg 100/g fish body mass) injection. A significant decrease of serum glucose content was observed along with the depletion of hepatic glycogen content at the 12-h time point following insulin injection. Hepatic gene expression analysis revealed that the infusion of insulin significantly increased the expression of glucose transporters (GLUTs), GLUT1b and GLUT2. The transcription of gluconeogenesis-related genes, fructose-1,6-bisphosphatase 1a and glucose-6-phosphatase a catalytic subunit tandem duplicate 2, was significantly decreased at the 12-h time point, while the expression of glycolysis-related genes, phosphofructokinase liver b and pyruvate kinase L/R, was significantly up-regulated at the 24-h time point post-insulin injection. Meanwhile, the expression of glucokinase significantly increased immediately following insulin administration. The expression of glycogen synthase liver type significantly decreased at the 12-h time point, while the expression of glycogen phosphorylase liver type was significantly elevated at the 24-h time point following insulin injection. These results suggested that insulin could affect glucose metabolism through regulating gluconeogenesis,

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# 研究背景

石斑鱼，是一种肉食性鱼类，生长速度快，具有重要的市场经济价值。研究表明，杂交石斑鱼具有较低的葡萄糖代谢速率，因此它餐后会处于高血糖状态。

胰岛素是一种肽类激素，在维持葡萄糖稳态中扮演着重要角色。研究表明，大多数鱼类在被胰岛素处理后，会处于低血糖状态，且肝糖原合成减少，肌糖原合成增加。胰岛素对糖代谢的相关研究很多，但胰岛素对糖代谢相关基因的调控作用却还有待探究。

本研究以杂交石斑鱼为研究对象，旨在探究石斑鱼被胰岛素处理后，胰岛素对血糖，糖原合成及糖代谢相关基因表达量的影响。

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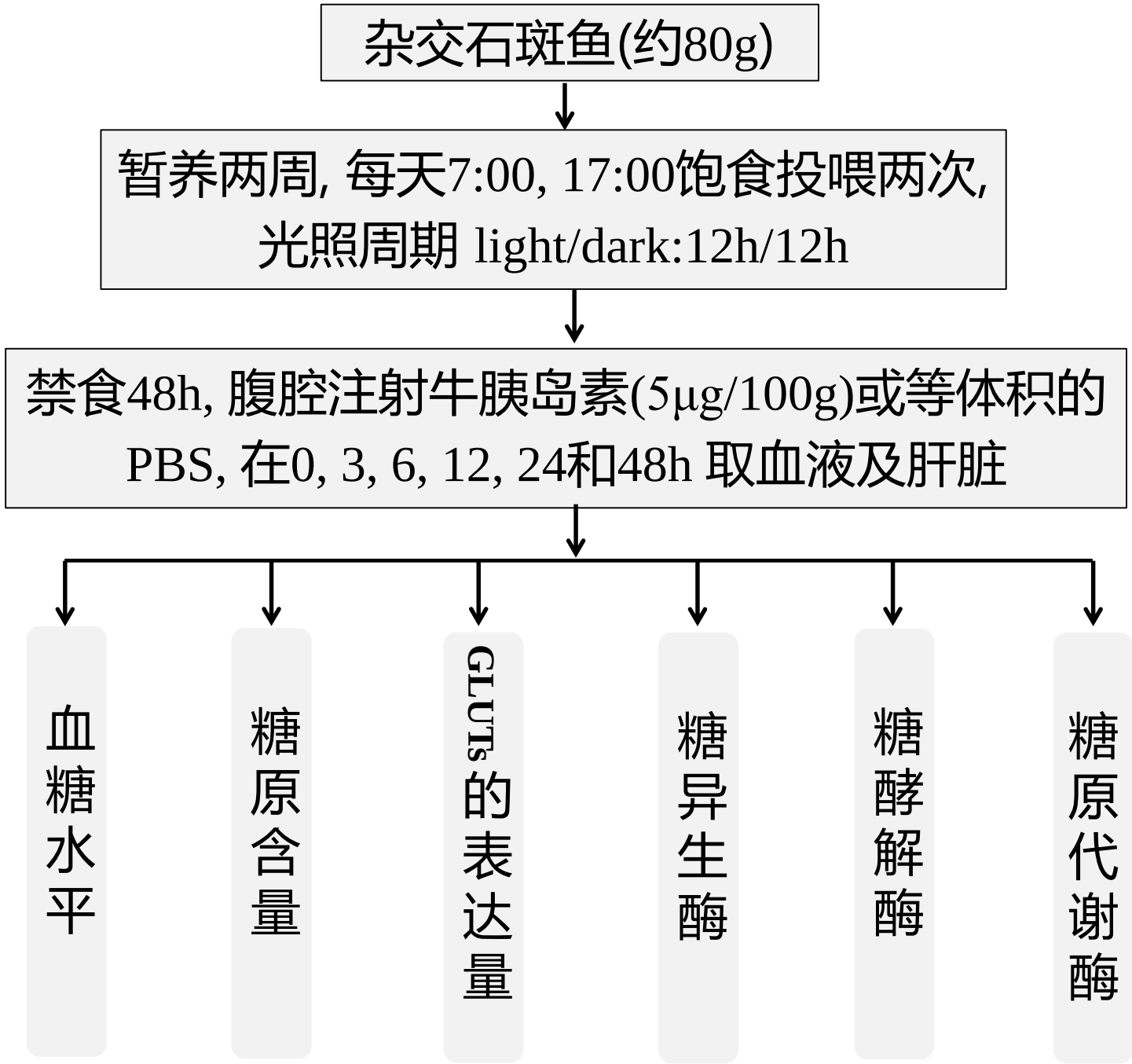
# 材料与方法


杂交石斑鱼(约80g)
暂养两周, 每天7:00, 17:00饱食投喂两次, 光照周期 light/dark:12h/12h
禁食48h, 腹腔注射牛胰岛素(5μg/100g)或等体积的PBS, 在0, 3, 6, 12, 24和48h 取血液及肝脏
血糖水平
糖原含量
GLUTs的表达量
糖异生酶
糖酵解酶
糖原代谢酶

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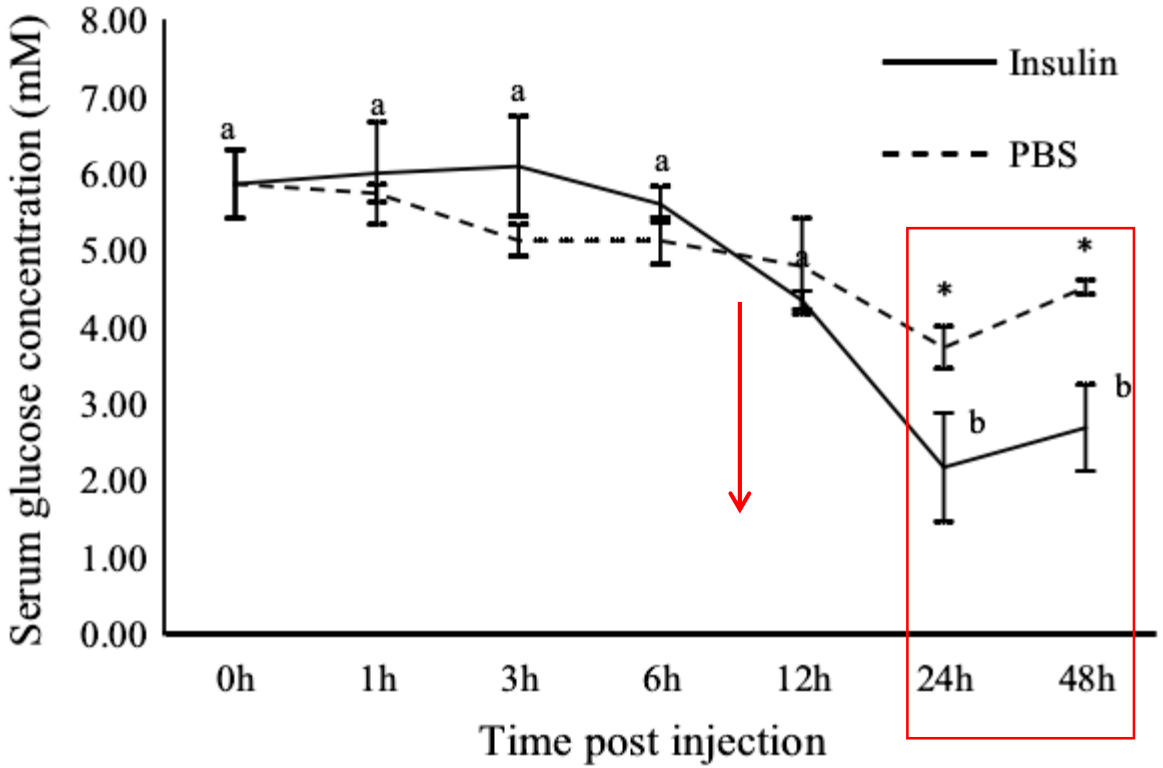
# 结果与分析

**Table 1** Primers used in the present study

| Gene | Sequence (5′–3′) | | GenBank number |
|---|---|---|---|
| | Forward | Reverse | |
| Glucose transporter 1b (GLUT1b) | TGGACTCAGGAAAGCAAG | GGAGAAGGAGCCAAAGAT | KY656466 |
| Glucose transporter 2 (GLUT2) | TGTTCTGCTTTTCGGCTTC | CAGTTCCGCATTGTCTATG | KY656467 |
| Glucose-6-phosphatase (G6PCa2) | CACAGTCCGTCCTCACAT | GCAAAACAGCGTCCATAA | MH213269 |
| Fructose-1,6-bisphosphatase 1a (FBP1a) | CGACCAAGGAGTCATTGAC | GATACCAGCTTTACGCACA | MH213268 |
| Phosphoenolpyruvate carboxykinase 1 (PEPCK1) | GCCGCTCAAAAAACCCTT | CCTCCTCCTTGGCAATCC | MH213267 |
| Glucokinase (GK) | TGGGTTTTACCTTCTCCTT | AGTCCCCTCGTCTCTTGAT | MH213270 |
| Phosphofructokinase liver b (PFKLb) | AAACGCCCATGCAAACTAC | CAACCTCTCTGACAGCCAC | MH213271 |
| Pyruvate kinase L/R (PKLR) | ATTTCTCTCACGGCTCACA | CCTCCACTTTCCCTTTCAC | MH213272 |
| Glutamine synthetase (GS2) | CAGGTTTTGGCTGCTTTATG | CTCTCAGTTCGGTTCCGTTG | MH213273 |
| Glycogen phosphorylase liver type (PYGL) | TCACAGAATACGCCACCGAG | CATTTTTGAGACACCCACAG | MH213274 |
| β-actin | CTCTGGGCAACGGAACCTCT | GTGCGTGACATCAAGGAGAAGC | AY510710 |




血糖水平

**Fig. 1** Serum glucose (mM) in hybrid grouper following insulin (*solid lines*) and phosphate-buffered saline (*PBS*) (*dashed lines*) injection. Sampling was at the 0, 1, 3, 6, 12, 24 and 48-h time points post-injection. Values (mean ± SEM) shown by *bars* that have the *same letter* are not significantly different ($P > 0.05$; Tukey's test) among treatments ($n = 3$). *Asterisk* indicates a significant difference ($P < 0.05$; Student's *t*-test) at the same time point between the insulin and PBS treatment ($n = 3$)

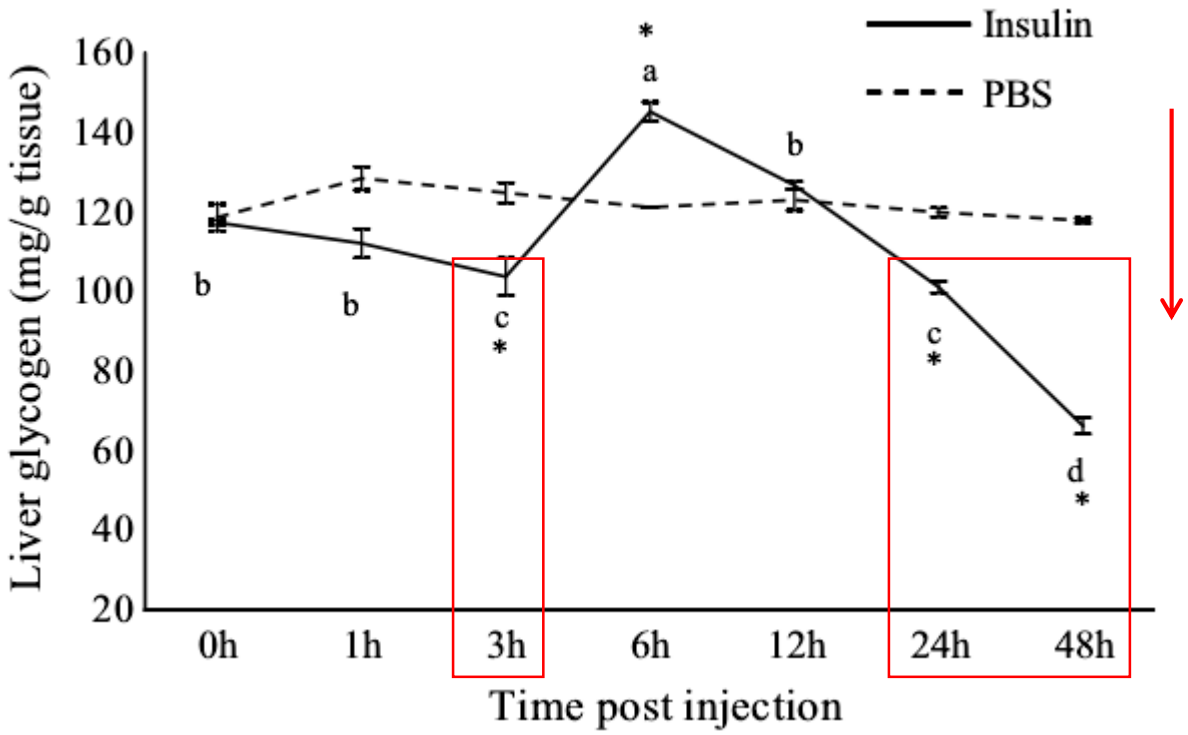
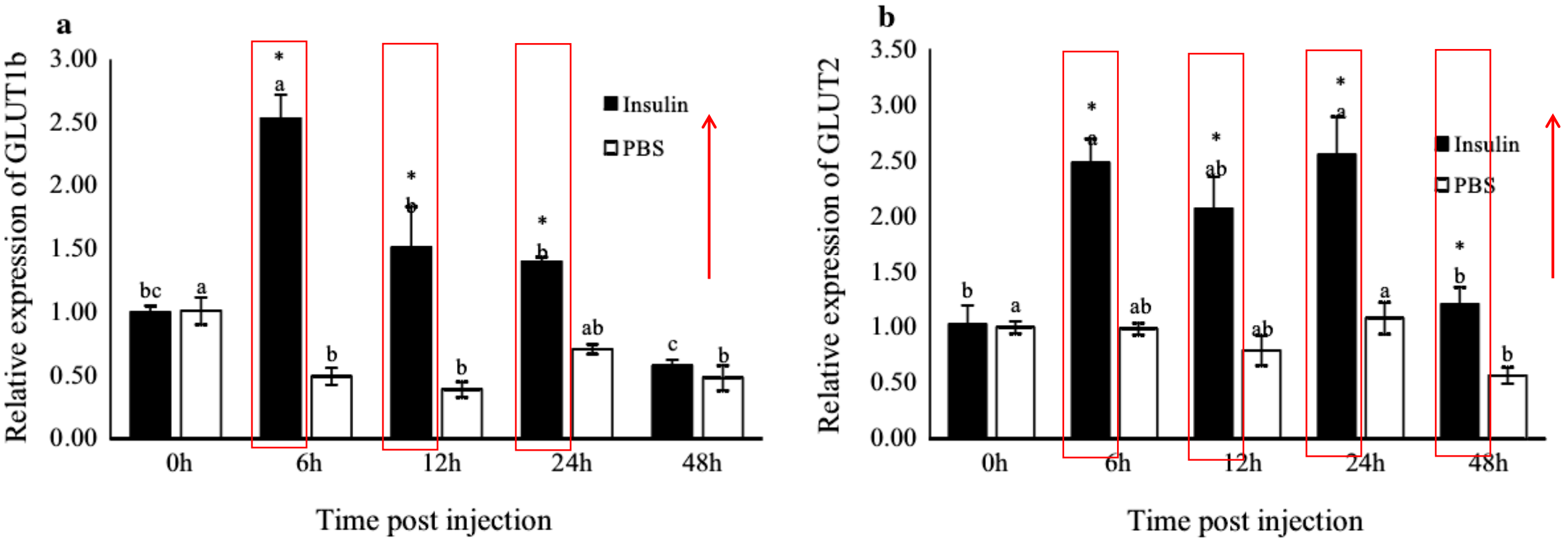



**Fig. 2** Glycogen content (mg/g tissue) in the liver of hybrid grouper following insulin (*solid lines*) and PBS (*dashed lines*) injection. Sampling at 0, 1, 3, 6, 12, 24 and 48-h time points post-injection. Values (mean ± SEM) shown by *bars* that have the *same letter* are not significantly different ($P > 0.05$; Tukey's test) among treatments ($n = 3$). *Asterisk* indicates a significant difference ($P < 0.05$; Student's *t*-test) at the same time point between the insulin and PBS treatment ($n = 3$)

从总体趋势来看，肝糖原含量是下降的，说明insulin的作用就是增加糖原的消耗.

# GLUTs的表达量



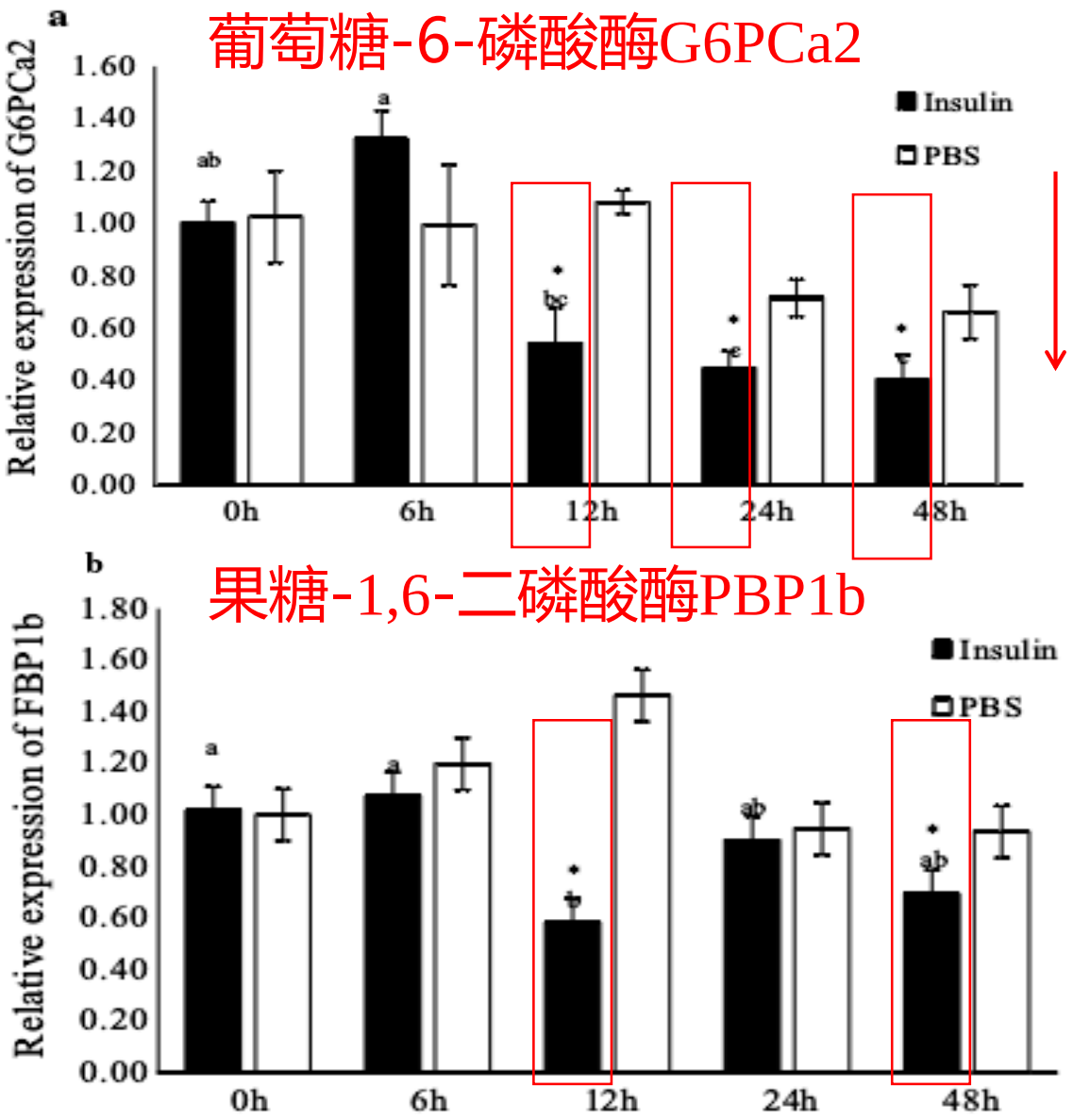

GLUT1b

图3

GLUT2

Insulin能通过促进GLUT1b，GLUT2的表达，来促进葡萄糖向血液输出。

# 糖异生相关基因的表达量



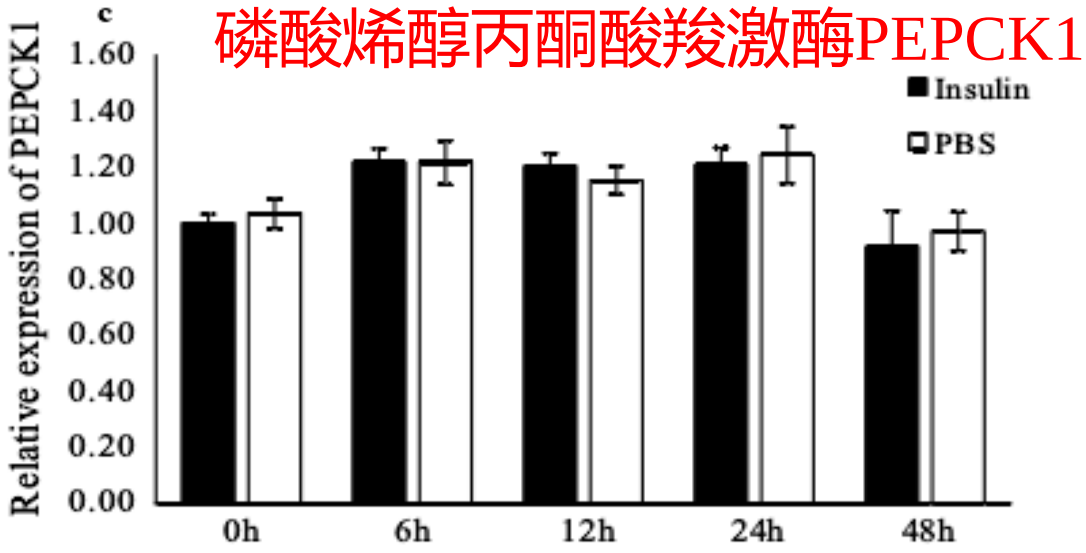
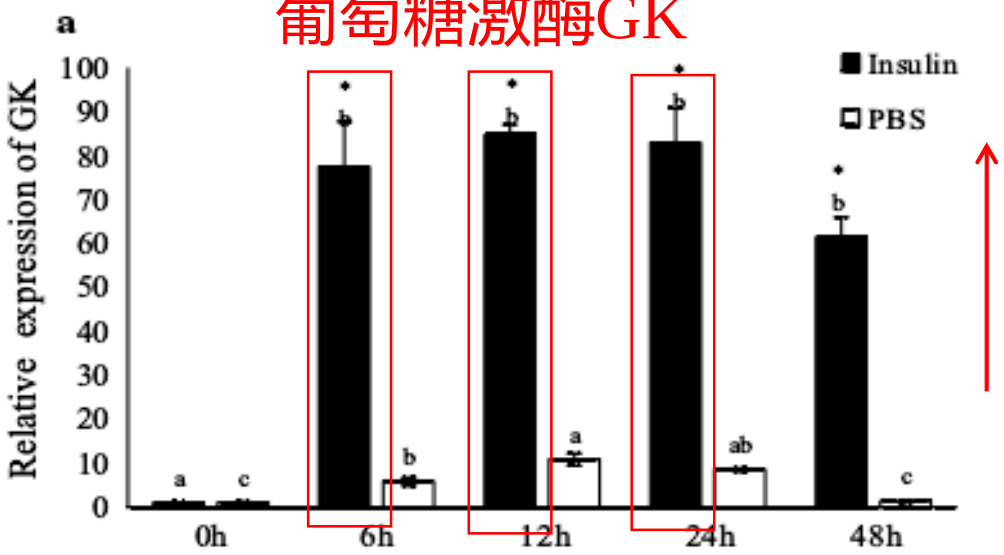




图4

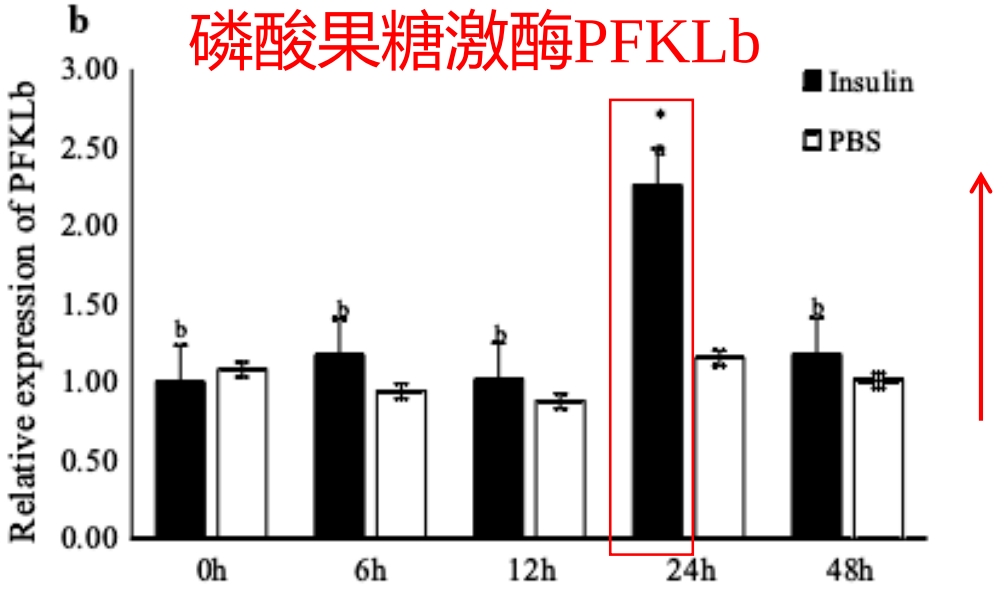
Insulin通过抑制葡萄糖-6-磷酸酶G6PCa2和果糖-1,6-二磷酸酶PBP1b的表达来调控葡萄糖代谢.

# 糖酵解相关基因的表达量

葡萄糖激酶GK




磷酸果糖激酶PFKLb




丙酮酸激酶PKLR

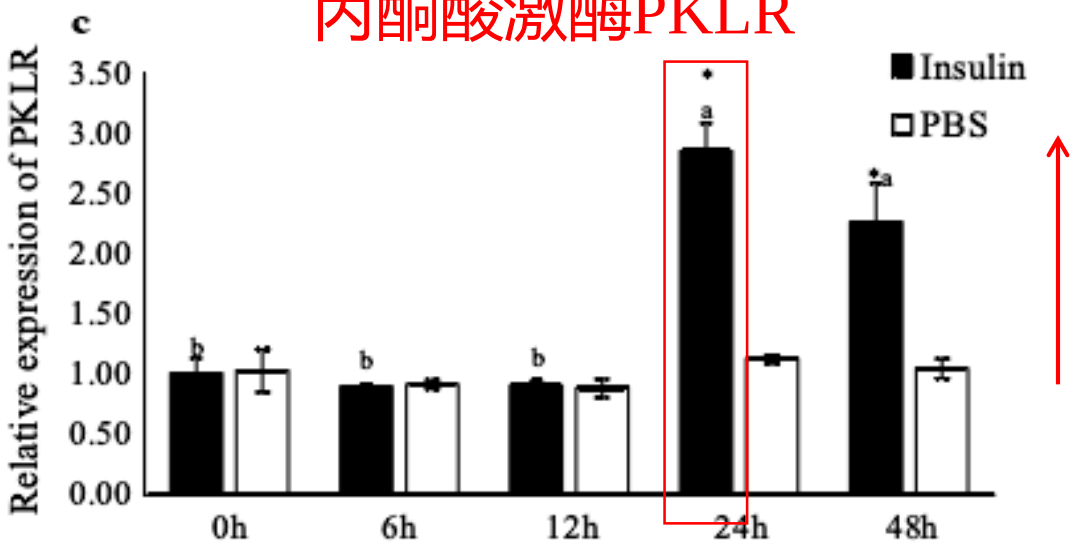



图5

Insulin能通过促进糖酵解相关基因的表达来调控葡萄糖代谢.

# 糖原代谢相关基因的表达量

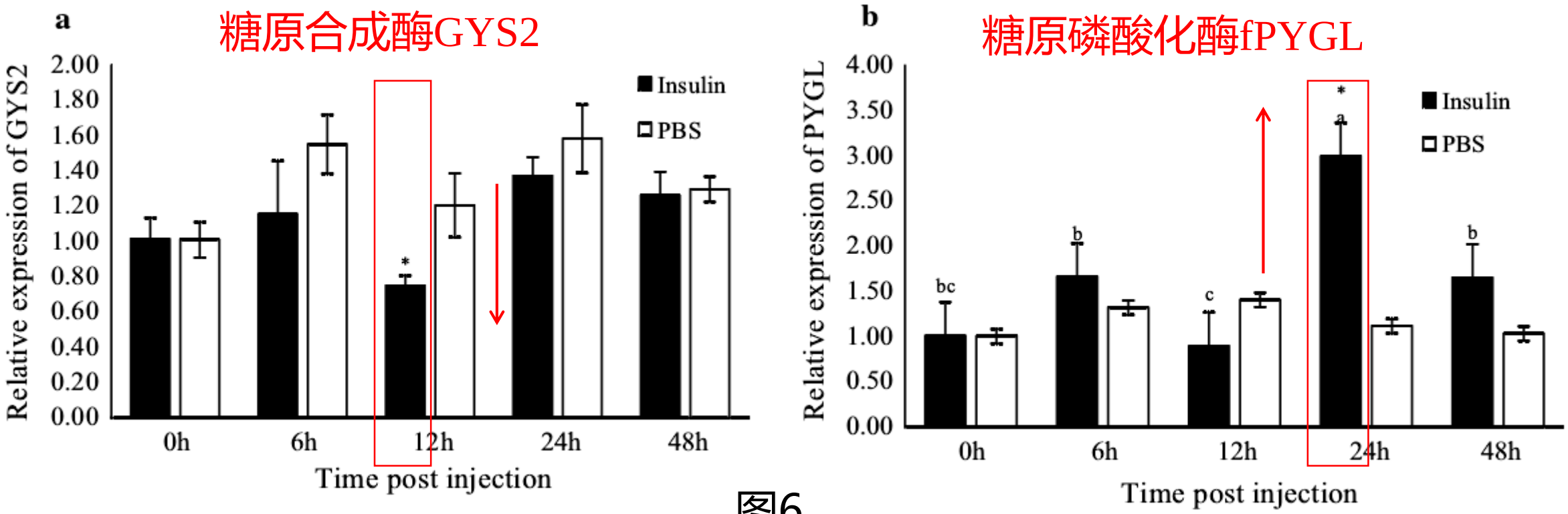



图6

注射insulin后，糖原合成酶GYS2表达量下降且糖原磷酸化酶fPYGL表达量，这可能与糖原含量降低有关。

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# 总结

1.腹腔注射胰岛素后，胰岛素可造成肝糖原含量及血糖水平的下降。

2.腹腔注射胰岛素后，葡萄糖转运蛋白GLUT1b和GLUT2表达量增加，表明胰岛素可对GLUT1b和GLUT2进行调控。

3. GLUT1b和GLUT2的高表达表明机体对葡萄糖的糖转运能力增强，而血糖下降可能与糖酵解相关基因的上调和糖异生相关基因的下调有关。

# 请各位老师批评指正