Objective To investigate the regulation of insulin sensitivity in liver cells by androgen signaling. Methods: Eleven adult female C57BL/6 mice were injected daily with testosterone (group T) for 24 weeks. Ten control mice were injected with sesame oil only (group Con). HepG2 liver cells were firstly pretreated with different doses of testosterone (10-9-10-5 mol/L) for 0-36 h or with 10-7 mol/L testosterone for 0-96 h followed by once stimulation with 100 nmol/L insulin for 15 min. Lately HepG2 cells were pretreated with 10-7 mol/L testosterone for 36 h followed by stimulation with 100 nmol/L insulin for 15 min, and then restimulated with 100 nmol/L insulin for 15 min at 4 h and 6 h interval, respectively. Phosphorylation and protein expression of Akt and GSK3β from C57BL/6 mice liver tissues and HepG2 cells were analyzed by western blot. Results: 24 weeks treatment with testosterone decreased phosphorylation of Akt and GSK3β in C57BL/6 adipose and liver tissues (43.10±3.23% vs 77.12±6.70%, 14.72±6.74% vs 82.31±2.00%, respectively, P<0.05). Pretreatment with 10-8~10-6 mol/L testosterone within 36 h obviously increased phosphorylation of Akt and GSK3β (P<0.05), whereas pretreatment with 10-5 mol/L within 36 h or with 10-7 mol/L for 96 h did not influence phosphorylation of Akt and GSK3β compared with control group (P>0.05). Pretreatment with 10-7 mol/L testosterone for 36 h followed by insulin stimulation and restimulation after 6 h interval obviously decrease phosphorylation of Akt and GSK3β (P<0.05). Conclusion androgen signaling might contribute to the insulin resistance in liver cells.