OBJECTIVE——Insulin represses the expression of gluconeo- genic genes at the mRNA level, but the hormone appears to have only weak inhibitory effects in vivo.The effect was modest compared with its inhibitory effect on net hepatic glycogenolysis, occurred within 30 min, and was associated with a marked decrease in hepatic fat oxidation, increased liver fructose 2,6–bisphosphate level, and reductions in lactate, glycerol, and amino acid extraction. The aims of this study were 1) to determine the maximal physiologic effect of insulin, 2) to determine the relative importance of its effects on gluconeogenic regulatory sites, and 3) to correlate those changes with alter- ations at the cellular level.No further diminu- tion in gluconeogenic flux to G6P occurred over the remaining 4.5 h of the study, despite a marked decrease in PEPCK content, suggesting poor control strength for this enzyme in gluconeo–genic regulation in canines.In the healthy state, small increases (twofold) in the plasma insulin level can result in near–complete inhibition of the net contribution of glycogen to hepatic glucose production (HGP) (1).RESEARCH DESIGN AND METHODS——Conscious 60–h fasted canines were studied at three insulin levels (near basal, 4 , or 16 ) during a 5–h euglycemic clamp.RESULTS——Insulin reduced gluconeogenic flux to glucose–6- phosphate (G6P) but only at the near–maximal physiological level (16 basal).Initially decreased hepatic lactate extraction is important, and later reduced gluconeogenic precursor availability plays a role.Pancreatic hormones were controlled using somatostatin with portal insulin and glucagon infusions.Glucose metabolism was assessed using the arteriovenous difference technique, and molecular signals were assessed.CONCLUSIONS——Gluconeogenic flux can be rapidly inhibited by high insulin levels in canines.Diabetes 58: 2766–2775, 2009 Hepatic glycogen metabolism in vivo is ex- tremely sensitive to the effects of insulin.On the other hand, the effects of insulin on hepatic gluconeogenesis are less potent, are more complex, and occur through multiple mechanisms.Corresponding author: Dale S. Edgerton, dale.edgerton@vanderbilt.edu.Published ahead of print at http://diabetes.diabetesjournals.org on 15 September 2009.The direct inhibitory effect of insulin on the From the Vanderbilt University Medical Center, Nashville, Tennessee.Changes in PEPCK appear to have little or no acute effect on gluconeogenic flux.This effect has been as- cribed to activation of glycogen synthesis (2).DOI: 10.2337/db09–0328.The costs of publication of this article were defrayed in part by the payment of page charges.Received 17 March 2009 and accepted 8 August 2009.