Effects of Insulin on the Metabolic Control of Hepatic Gluconeogenesis in vivo

Dale S. Edgerton, Christopher J. Ramnanan, Carrie A. Grueter, Kathryn M. S. Johnson, Margaret Lautz, Doss W. Neal, Phillip E. Williams, Alan D. Cherrington

Vanderbilt University Medical Center, Nashville, Tennessee

Corresponding Author:
Dale S. Edgerton, PhD
E-mail: dale.edgerton@vanderbilt.edu

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org

Submitted 17 March 2009 and accepted 8 August 2009.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.
**Objective.** Insulin represses the expression of gluconeogenic genes at the mRNA level, but the hormone appears to have only weak inhibitory effects *in vivo*. The aims of this study were to determine 1) the maximal physiologic effect of insulin, 2) the relative importance of its effects on gluconeogenic regulatory sites and to 3) correlate those changes with alterations at the cellular level.

**Research Design and Methods.** Conscious 60-h-fasted dogs were studied at three insulin levels (near basal, 4x, or 16x) during a 5-h euglycemic clamp. Pancreatic hormones were controlled using somatostatin with portal insulin and glucagon infusions. Glucose metabolism was assessed using the arterio-venous difference technique and molecular signals were assessed.

**Results.** Insulin reduced gluconeogenic flux to G6P but only at the near maximal physiological level (16x basal). The effect was modest compared to its inhibitory effect on net hepatic glycogenolysis, it 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. No further diminution 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 gluconeogenic regulation in the dog.

**Conclusions.** Gluconeogenic flux can be rapidly inhibited by high insulin levels in the dog. Initially decreased hepatic lactate extraction is important and later reduced gluconeogenic precursor availability plays a role. Changes in PEPCK appear to have little or no acute effect on gluconeogenic flux.
Hepatic glycogen metabolism in vivo is extremely sensitive to the effects of insulin. In the healthy state, small increases (2-fold) in the plasma insulin level can result in near complete inhibition of the net contribution of glycogen to hepatic glucose production (HGP) (1). This effect has been ascribed to activation of glycogen synthesis (2). On the other hand, the effects of insulin on hepatic gluconeogenesis are less potent, more complex and occur through multiple mechanisms. The direct inhibitory effect of insulin on the transcription and activity of key hepatic gluconeogenic enzymes through FOXO-1 phosphorylation, including phosphoenolpyruvate carboxykinase (PEPCK) (3; 4), is well established. Further, insulin inhibits the secretion of glucagon, a known activator of gluconeogenesis (5), thereby bringing about an indirect inhibitory effect on the process in the liver. In addition, insulin inhibits lipolysis (6), which reduces circulating glycerol and non-esterified free fatty acid (NEFA) levels. Glycerol is an important gluconeogenic (GNG) precursor and NEFA provide energy for gluconeogenesis (7). Net hepatic lactate uptake is reduced by insulin through several mechanisms. First, the hormone influences substrate cycling through the glycolytic / GNG pathways via allosteric and phosphorylation mediated changes in the activity of key enzymes (e.g. bifunctional enzyme and pyruvate kinase (PK)) (4; 8). Second, hepatic lactate flux is regulated by insulin secondary to its inhibitory effect on lipolysis (e.g. via reduced citrate which results in increased phosphofructokinase-1 (PFK-1) activity) (4; 8; 9). Insulin decreases net amino acid release from muscle via inhibition of proteolysis and potentially by increased protein synthesis (10), although the effect of reduced gluconeogenic amino acid precursor availability is offset to some degree by an increase in hepatic amino acid transport (11). Finally, evidence in the rodent suggests that hepatic gluconeogenesis may also be inhibited as the result of insulin action in the hypothalamus. It is postulated that brain insulin action increases vagal transmission, thereby increasing the phosphorylation of STAT3 in the liver and in turn reducing PEPCK and glucose-6-phosphatase (G6Pase) transcription (12-14).

Assessment of gluconeogenesis in vivo is complicated by the sensitive effects of insulin on hepatic glycogen metabolism. Since higher insulin concentrations are required to suppress gluconeogenesis than to inhibit glycogenolysis or increase glycogen synthesis (15; 16), gluconeogenically derived glucose-6-phosphate (G6P) can be diverted into hepatic glycogen even during mild hyperinsulinemia. As a result, redirection of the product of GNG flux into hepatic glycogen can decrease gluconeogenesis per se, in the absence of a fall in GNG flux to G6P (1; 17).

Despite the numerous mechanisms by which insulin can inhibit the gluconeogenic process, acute physiologic increases in insulin have minimal impact on GNG flux in humans and large animals (1; 15; 17-20). Much of what is known about the regulation of gluconeogenesis is derived from in vitro studies on tissues or cells which lack important inputs for GNG control (e.g. gluconeogenic precursor load, NEFA availability, neuronal transmission, glycogen, hormonal milieu, etc). Likewise, many studies of GNG regulation have been performed in the rodent, and it is not clear how control of the process may differ in those species compared to larger animals (e.g. basal rates of gluconeogenesis are 5-10 higher in the rat and mouse than in man or dog; glycogen stores are completely depleted during fasting in the rodent but not in man or dog).
Given the difficulty in detecting an inhibitory effect of insulin on GNG flux in vivo, conditions were optimized in the present study to allow such an effect to be seen. First, the dogs were fasted for 60-h to increase the percent contribution of gluconeogenesis to HGP. In addition, since we previously failed to observe any suppression of GNG flux in overnight fasted dogs when the level of insulin was increased 4-fold (20), we examined both 4- and 16-fold increases in insulin. Finally, insulin was elevated for 5-h to provide sufficient time for changes in insulin signaling to affect gene transcription, and for the latter to translate into substantial effects on gluconeogenic enzyme levels and activities. In order to focus on the effects of insulin, glucagon and glucose were clamped at basal levels. In addition, liver biopsies were taken at the end of each study to allow the effects of insulin on molecular signaling and gene transcription to be correlated with alterations in whole body metabolic flux rates.

**RESEARCH DESIGN AND METHODS**

**Animal care and surgical procedures.** Sixteen conscious mongrel dogs of either sex were studied after a 60-h fast. Housing and diet have been described previously (1). The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. All dogs underwent a laparotomy two weeks before the experiment in order to implant portal vein infusion catheters into the jejunal and splenic veins, sampling catheters into the femoral artery and portal and hepatic veins, and ultrasonic flow probes (Transonic Systems, Ithaca, NY) around the hepatic artery and portal vein, as described elsewhere (1). All dogs studied were healthy, as indicated by: 1) leukocyte count <18000/mm³, 2) a hematocrit > 35%, 3) a good appetite, and normal stools.

**Experimental Design.** Animals were allowed to rest quietly in a Pavlov harness for 60 min before the experiments started. Each study consisted of a basal period (-40 to 0 min) and an experimental period (0 to 300 min). Somatostatin (0.8 µg/kg/min; Bachem, Torrance, CA) was infused (0 to 300 min) to inhibit endogenous pancreatic hormone secretion. During the same period intraportal infusions of glucagon (0.5 ng/kg/min; Lilly, Indianapolis, IN) and insulin (300, 1200, or 5000 µU/kg/min; Lilly) were given in the control (n=5), 4x (n=5) or 16x (n=6) groups, respectively. Previously, when the plasma glucose level was maintained at basal levels by titration of intraportal insulin in the 60-h fasted dog, the insulin requirement was 215±24 µU/kg/min (21). Therefore, in the present study, insulin was infused in the control group at a rate slightly above basal. Glucose was infused intravenously to maintain euglycemia.

Immediately after the final sampling time each animal was anesthetized and three sections of liver lobes were freeze clamped in situ and stored at -70°C as previously described (1). All animals were then euthanized and the correct positions of the catheter tips were confirmed.

**Analytical procedures.** Hematocrit; plasma glucose, glucagon, insulin, cortisol, and NEFA; blood alanine, glycine, serine, threonine, lactate, glutamine, glutamate, glycerol and β-hydroxybutyrate (β-OHB) concentrations were determined as previously described (1). RNA extraction, cDNA synthesis, real-time PCR, SDS-PAGE and Western blotting procedures were performed by standard methods, details of which are provided in the supplemental materials which are available in the online appendix at http://diabetes.diabetesjournals.org. Fructose-2,6-bisphosphate (F2,6P2) levels and PK
activity were determined as described in the supplemental materials. **Calculations.** Net hepatic substrate balances were calculated with the A-V difference method using the formula: 

\[ \text{NHB} = \text{Load}_{\text{out}} - \text{Load}_{\text{in}} \]

where \( \text{Load}_{\text{out}} = [H]*HF \) and \( \text{Load}_{\text{in}} = [A]*AF + [P]*PF \) and where \([H]\), \([A]\), and \([P]\) are the substrate concentrations in hepatic vein, femoral artery, and portal vein blood or plasma, respectively, and \(HF\), \(AF\) and \(PF\) are the blood or plasma flow in the hepatic vein, hepatic artery and portal vein, respectively, as determined by the ultrasonic flow probes. With this calculation, a positive value represents net output by the liver, while a negative value represents net hepatic uptake. Plasma glucose values were multiplied by 0.73 to convert them to blood glucose values as validated elsewhere (20). Net hepatic fractional extraction was calculated by dividing net hepatic substrate balance by hepatic substrate load. Non-hepatic glucose uptake was calculated as the glucose infusion rate plus net hepatic glucose balance, with changes in the glucose mass accounted for when deviations from steady-state were present. The approximate insulin and glucagon levels in plasma entering the liver sinusoids were calculated using the formula 

\[ [A] * \%AF + [P] * \%PF \]

where \([A]\) and \([P]\) are arterial and portal vein hormone concentrations, respectively, and \(\%AF\) and \(\%PF\) are the respective fractional contributions of arterial and portal flow to total hepatic blood flow.

Gluconeogenesis is the synthesis and subsequent hepatic release of glucose from non-carbohydrate precursors. Because carbon produced from flux through the gluconeogenic pathway can also be stored in glycogen, we make a distinction between gluconeogenesis and GNG flux to G6P. Hepatic GNG flux to G6P was determined by summing net hepatic uptake rates of gluconeogenic precursors (alanine, glycine, serine, threonine, glutamine, glutamate, glycerol, lactate and pyruvate); these rates were divided by two to account for the incorporation of three carbon precursors into the six-carbon glucose molecule. Glycolytic flux was estimated by summing the net hepatic output rates (when such occurred) of the substrates noted above (in glucose equivalents) and hepatic glucose oxidation (GO). In earlier studies GO was 0.2±0.1 mg/kg/min even when the concentrations of circulating insulin, glucose and NEFA varied widely (22; 23). Since GO did not change appreciably under conditions similar to the present study GO was assumed to be constant (0.2 mg/kg/min). Net hepatic glycolyotic flux was estimated by subtracting GNG flux from the sum of net hepatic glucose balance and glycolytic flux. A positive number therefore represents net glycogen breakdown whereas a negative number indicates net glycogen synthesis.

The assumptions related to using the A-V difference technique for assessing GNG flux to G6P should be considered. Ideally GNG flux would be calculated using unidirectional hepatic uptake and output rates for each substrate, but this would be difficult, as it would require the simultaneous use of multiple stable isotopes which could themselves induce a mild perturbation of the metabolic state, therefore net hepatic balance was used instead. There is little or no production of gluconeogenic amino acids or glycerol by the liver so in this case the compromise is of little consequence. This may not be the case for lactate, however. The estimate of GNG flux to G6P will only be quantitatively accurate if lactate flux is unidirectional at a given moment (i.e. either in or out of the liver). In a given cell this is a reasonable assumption in light of the reciprocal control of gluconeogenesis and glycolysis (4). Spatial separation of metabolic pathways may exist, however, so that predominantly gluconeogenic periportal and glycolytic perivenous hepatocytes (24) could
simultaneously take up and release lactate, respectively. Total GNG flux will be underestimated to the extent that this occurs, and to the extent that intrahepatic precursors (GNG amino acids) contribute to the process. The method also assumes that there is 100% conversion of gluconeogenic precursors taken up by the liver into G6P (they are not oxidized or used in the synthesis of proteins or fatty acids). The errors due to these assumptions are difficult to assess, but appear to be small and in fact are offsetting. Results obtained using the A-V difference technique described here were previously compared to results obtained with independent gluconeogenic measurements that are not subject to these assumptions and they were similar (1; 25), suggesting that these assumptions appear reasonable.

It should be noted that our estimates of gluconeogenesis and glycogenolysis relate solely to the liver. In a previous study net renal glucose balance was close to zero in 60-h fasted dogs indicating that in a net sense the liver is the only source of glucose in such animals (21). To the extent that the kidney makes an absolute contribution to whole body glucose production, we would slightly underestimate whole body gluconeogenic flux.

Statistical analysis. Statistical comparisons were carried out using two-way repeated measure ANOVA (group x time) (SigmaStat). One-way ANOVA comparison tests were used post hoc when significant F ratios were obtained. Significance was determined as P<0.05.

RESULTS

Hormone levels. Plasma insulin levels did not increase appreciably in the control group, whereas they increased an average of 4- and 16-fold in the 4x and 16x groups, respectively (Fig. 1). The plasma arterial and hepatic sinusoidal glucagon and arterial cortisol levels remained basal and were similar over time and between groups (not shown).

Fat metabolism. Hepatic NEFA uptake and oxidation play a role in the regulation of hepatic gluconeogenesis in vivo as does the load of glycerol reaching the liver. In the control group, the arterial plasma NEFA level fell by ~25% during the experimental period, while the glycerol level did not change significantly (~10%; Table 1). There was a rapid fall in the arterial NEFA levels in the 4x and 16x insulin groups (reduced by 58 and 75%, respectively, at 30 min; ↓85 and 94% during the last hour). Glycerol levels also decreased in the two hyperinsulinemic groups (↓45 and 43% at 30 min; ↓40 and 65% during the last hour, respectively). These changes indicate rapid inhibition of lipolysis and increased re-esterification of fatty acids. In the control group, net hepatic NEFA uptake tended to decrease slowly over time (↓30% by the last hour) whereas in the 4x and 16x groups, net hepatic NEFA uptake decreased quickly (↓61 and 81%, respectively, at 30 min) and was markedly reduced (↓85 and 95%, respectively) by the last hour of the study (Table 1). Hepatic NEFA oxidation was also reduced by hyperinsulinemia, as indicated by marked reductions in β-OHB production in both hyperinsulinemic groups (Table 1).

Gluconeogenic precursor availability and hepatic uptake. Lactate, glycerol and alanine make the greatest quantitative contributions to GNG flux to G6P (52, 12 and 20% respectively, during the basal state), with the other amino acids accounting for the remainder. In the control group there were no changes in the level, net hepatic fractional extraction (NHFX), or net hepatic uptake of lactate over time (Table 2). There were rapid and sustained decreases in the NHFX of lactate in the 4x and 16x groups (↓40 and 50%, respectively, at 30 min) which persisted until the end of the study. Arterial lactate levels tended to rise in the 4x group
Effects of insulin on hepatic gluconeogenesis and increased even more substantially in the 16x group (↑16% and 84%, respectively, by the last hour). Therefore, as a result of these somewhat offsetting effects, net hepatic lactate uptake was reduced by ~50% (Δ-3 to -4 µmol/kg/min) at 30 min in both groups, but was only reduced by 33 and 23%, respectively, during the last hour.

Net hepatic glycerol uptake fell rapidly (↓31, 57 and 54% [Δ-0.7 to -1 µmol/kg/min]) by 30 min in the control, 4x and 16x groups, respectively) in parallel to changes in glycerol availability (Table 1). By the last hour of the study, rates of net hepatic glycerol uptake were reduced by 25, 42, and 68%, respectively.

In the control and 4x groups there were decreases in blood alanine level which offset increases in alanine NHFX so that net hepatic alanine uptake did not change significantly over time (Table 3). In the 16x group, there was a progressive decrease in the alanine level (↓50% by the end of the study) but the NHFX of alanine did not increase enough to offset this fall, therefore net hepatic alanine uptake was reduced by one third during the last hour of the study. The average summed gluconeogenic amino acid (alanine, serine, glycine, threonine, glutamate and glutamine) levels followed a similar pattern of decline as alanine (reduced by 16, 36 and 44% in the control, 4x and 16x groups, respectively) (Table 2). The NHFX of gluconeogenic amino acids increased from basal in the control and 4x groups (2.1- and 2.4-fold, respectively, during the last hour) but did not change in the 16x group. As a result, net hepatic gluconeogenic amino acid uptake increased by 26 and 38% (Δ1.4 to 1.7 µmol/kg/min) in the control and 4x groups, respectively, whereas in the 16x group there was a slow decline (↓44% by the last hour; Δ-2.5 µmol/kg/min) which paralleled amino acid availability.

Liver and non-hepatic glucose fluxes. Net hepatic glucose output (NHGO) was similar during the basal period in the three groups (1.6 to 2.0 mg/kg/min; Figure 2). By the last hour, NHGO was 1.3±0.2 mg/kg/min in the control group. In the 4x and 16x groups, hyperinsulinemia had suppressed NHGO by 50% at 30 min and by the last hour had switched the liver to net hepatic glucose uptake (0.2±0.1 and 0.9±0.3 mg/kg/min in the two groups, respectively). Euglycemia was maintained by glucose infusion (reaching 0.5±0.2, 6.6±1.0 and 15.5±1.5 mg/kg/min, in the control, 4x and 16x groups, respectively, during the last hour; Table 3). The majority of the increase in glucose requirement in the 4x and 16x groups related to increases in muscle glucose uptake (non-hepatic glucose uptake was 1.8±0.3, 6.4±1, and 14.7±1.6 mg/kg/min during the last hour in the three groups, respectively; Table 3).

Gluconeogenic and glycogenolytic flux rates. GNG flux to G6P did not change from basal in the control and 4x groups, whereas in the 16x group there was a rapid (30 min) and sustained decrease (↓35%; Δ-0.5 mg/kg/min; Table 2; Figure 3). In the control group, net hepatic glycogenolysis (NHGLY) decreased over time until it was almost completely inhibited by the end of the experiment. NHGLY decreased more rapidly in the 4x and 16x groups, and by the last hour of the study the liver had switched to net hepatic glycogen deposition (1.0±0.2 and 1.5±0.2 mg/kg/min, respectively; Figure 3).

Molecular effects of insulin. The effects of insulin treatment on targets of insulin receptor signaling were assessed in biopsies taken at the end of each experiment. Akt, a marker of activation of the PI3-K insulin-signaling pathway, was similarly activated in both the 4x and 16x groups, with the ratio of P-Ser473 Akt to total Akt increased 3.9- and 4.5-fold, respectively, compared to the control group (Figure 4A). In line with the changes in glycogen flux, the ratio of P-Ser9 GSK-3α to total GSK-3α increased 2.3- and 2.2-fold in the 4x and 16x
groups compared to control, and the ratio of
P-Ser21 GSK-3β to total GSK-3β increased
2.8- and 3.4-fold, respectively (Figure 4B).
The ratio of P-Ser256 FOXO1 to total
FOXO1 (a transcription factor involved in the
regulation of gluconeogenic enzymes,
including G6Pase and PEPCK) increased 3.0-
and 3.6-fold in the 4x and 16x groups,
respectively (Figure 4C) and analysis of total
FOXO1 levels in nuclear enriched fractions
detected reductions of 84 and 85%,
respectively, compared to the control group
(Figure 4C). While there were no significant
differences between the 4x and 16x groups for
Akt, GSK-3, or FOXO1 phosphorylation,
activation in the 16x group always tended to
be slightly greater compared to the 4x group.

G6Pase and glucokinase (GK) serve as
“gatekeepers” for hepatic glucose uptake and
release. G6Pase mRNA expression was
decreased by 60 and 80%, in the 4x and 16x
groups, respectively, compared to the control
group (Figure 4D). GK mRNA and protein
expression both increased in a step-wise
manner with increasing hyperinsulinemia:
mRNA levels increased 8.8- and 13.7-fold,
while protein levels in cytoplasmic-enriched
fractions increased 1.9- and 2.6-fold,
respectively (Figures 4E and 4F). Analysis of
 gluconeogenic markers revealed that
hyperinsulinemia decreased PEPCK mRNA
and protein levels by 90 and 60%,
respectively, with no differences between the
4x and 16x groups (Figure 4G and 4H).
Hepatic STAT-3 phosphorylation (activated
in the liver in response to central insulin
action (12)) was increased 3.5- and 5.2-fold
in the two groups, respectively.

F2,6P2 is a regulator of the glycolytic
and gluconeogenic pathways and PK
catalyzes the conversion of
phosphoenolpyruvate into pyruvate. At the
end of the study the hepatic concentrations of
F2,6P2 were 4.6±1.1, 11.6±1.7 and 26.9±3.3
nmol/g in the control, 4x and 16x groups,
respectively (Table 4). PK activities were
0.46±0.06, 0.70±0.09, and 0.82±0.08 U/mg
protein in the three groups, respectively
(Table 4).

**DISCUSSION**

The in vivo regulation of GNG flux by
insulin is complex and not fully understood.
In this study, conditions were optimized to
increase the likelihood of observing an acute
effect of insulin on the process. Dogs were
fasted 60-h to enhance gluconeogenesis,
insulin was elevated for 5-h to allow
sufficient time for transcriptional and
translational changes to occur, and two insulin
doses were administered (the highest of which
produced a maximal physiologic level). In
agreement with previous studies (20; 26),
GNG flux to G6P did not change significantly
over time in the control or 4x groups. At the
highest level of insulin, however, GNG flux
to G6P was reduced, but only by one third.
Further, this change occurred rapidly (within
30 min); presumably before an insulin
induced decrease in PEPCK activity was
possible. Thereafter, no further change in
GNG flux occurred, despite an eventual
marked decrease in PEPCK protein.

As expected, insulin signaling
pathways were activated with
hyperinsulinemia, as indicated by increased
phosphorylation of Akt, GSK-3 and FOXO1.
The balance between the activities of G6Pase
and GK determines glucose flux into and out
of the liver. Insulin reduced G6Pase mRNA
(and presumably the protein level) and
increased GK mRNA and GK protein levels
so that the ratio of G6Pase to GK expression
in the three groups corresponded inversely
with circulating insulin levels, reflecting the
shift towards net hepatic glucose uptake.

Net hepatic glycogenolysis accounted
for one third of the glucose produced by the
liver in the basal period (vs. two thirds
following an 18-h fast (1)), in agreement with
previous findings in 66-h fasted dogs in which
glycogenolysis was estimated to account for
41% of basal HGP using an independent technique and 23% using the A-V difference method (25). The decrease in HGP that occurred during hyperinsulinemia in the present study was primarily due to reduced net glycogenolysis. Insulin increased the phosphorylation of GSK-3, thereby inactivating it and facilitating activation of glycogen synthase (27). The rapid effect on glycogen metabolism occurred in a dose dependant manner, such that net glycogen synthesis was clearly evident after 5-h of hyperinsulinemia (delta 2.5 mg/kg/min) in the 16x group, even under euglycemic conditions. In the control group, an insulin infusion rate which was only modestly (~40%) above the basal endogenous insulin secretion rate (21) completely eliminated net glycogen breakdown by 5-h, demonstrating the exquisite sensitivity of liver glycogen metabolism to small increments in insulin.

GNG flux to G6P was rapidly reduced in the 16x group, decreasing near maximally by 30 min, although the effect was modest (delta from baseline 0.5 mg/kg/min). This initial decrease was primarily the result of a fall in net hepatic lactate uptake and was associated with the insulin induced inhibition of lipolysis. Human (28; 29) and dog (30) experiments have shown that plasma NEFA stimulate hepatic gluconeogenesis and that a fall in circulating NEFA causes a redirection of carbon flow within the liver from glucose to lactate output (9). It is likely that this results from increased glycolytic flux through PFK-1 due to reduced inhibition of the enzyme by citrate, a product of fat oxidation (4; 8). In the present study hyperinsulinemia led to robust, rapid, and dose dependant effects on fat metabolism (75% reduction in NEFA levels and net hepatic uptake and almost complete inhibition of hepatic fat oxidation in the 16x group at 30 min). In addition to NEFA mediated effects, the rapid effect of insulin on GNG flux to G6P was also probably explained by allosteric and covalent regulation of gluconeogenic and glycolytic enzymes. Insulin stimulates dephosphorylation of the bifunctional enzyme, activating the kinase and inactivating the phosphatase, thereby increasing F2,6P2 (4; 8). This leads to increased glycolytic flux through PFK-1 and to a lesser degree decreased gluconeogenic flux through fructose-1,6-bisphosphatase (4; 8). In addition, insulin activates PK by cAMP dependent and independent mechanisms and possibly by phosphorylation (4; 31), which allows for increased lactate formation from pyruvate. Indeed, in the 4x and 16x groups, respectively, we observed 2.5- and 5.8 fold increases in F2,6P2, 1.5- and 1.8-fold elevations in PK activity, although the time course of these changes is not known. Thus, the rapid decrease in GNG flux to G6P, and in particular net lactate uptake, can be explained by the direct and indirect effects of insulin.

The hepatic gluconeogenic precursor load and extraction are also determinants of GNG flux. In the 4x and 16x groups, inhibition of lipolysis resulted in decreased glycerol levels, and therefore the contribution of glycerol to GNG flux decreased. The inhibition of net hepatic lactate fractional extraction remained constant during hyperinsulinemia (~50%), however circulating lactate levels increased, especially in the 16x group (↑85%), as a result of reduced liver lactate uptake (32) and increased muscle and fat lactate production (33; 34). Net hepatic GNG amino acid fractional extraction increased quickly in the 4x group, to nearly twice the basal rate at 30 min, most likely due to insulin stimulation of hepatic amino acid transport (35). Therefore, despite decreased amino acid availability (probably due to reduced muscle protein turnover (10)), net hepatic GNG amino acid uptake increased enough to offset the reductions in net lactate and glycerol uptake in the 4x group, and thus GNG flux did not change. In the 16x group, although hepatic
Effects of insulin on hepatic gluconeogenesis

Extraction of alanine increased by 40% during hyperinsulinemia, a collective increase in GNG amino acid fractional extraction did not occur. Thus, decreased net hepatic uptake of lactate, glycerol and GNG amino acids all contributed to the fall in GNG flux in this group. The fall in lactate uptake predominated early while reduced amino acid uptake became more important by the end of the study.

PEPCK is considered to be a key gluconeogenic enzyme, and its mRNA levels are frequently used as an index of gluconeogenesis. PEPCK mRNA and protein expression were measured at the end of the study to determine their correspondence with GNG flux. Despite marked and equal suppression of PEPCK by insulin in the 4x and 16x groups (mRNA decreased by 90% and protein by 60%), GNG flux did not change in the 4x group, and there was only a 33% decrease in the 16x group. In addition, inhibition of GNG flux in the 16x group occurred within 30 min of the rise in insulin, and the degree of inhibition remained unchanged over the subsequent 4.5-h of hyperinsulinemia. Although PEPCK transcription can be reduced by insulin in vitro within minutes (36; 37), the enzyme’s activity is determined by its protein levels, which change much more slowly. Therefore, a decrease in PEPCK activity is unlikely to explain the rapid changes in GNG flux which we observed. Thus, while PEPCK transcription and translation were clearly regulated by insulin, the enzyme did not appear to be rate limiting for gluconeogenesis. Some studies in the rat have demonstrated control of gluconeogenesis and glucose production via inhibition of PEPCK with 3-mercaptopicolinate (38; 39) and it has been concluded that PEPCK is the rate-controlling enzyme for gluconeogenesis (40). On the other hand, studies using metabolic control analysis in isolated hepatocytes have suggested that a number of enzymes play important roles in controlling GNG flux, and that PEPCK displays weak control strength (41-43). Our results agree with those from studies in the transgenic mouse where a 70% reduction in PEPCK content resulted in only a 20% reduction in GNG flux (44) and from a very recent study in patients with T2DM where increased transcriptional expression of PEPCK did not account for increased gluconeogenesis (45). Thus, at least in the dog and human, PEPCK appears to contribute little to the hormonal regulation of gluconeogenesis.

Insulin action in the hypothalamus may also control hepatic gluconeogenesis (12-14), although this has only been demonstrated in the rodent. Activation of brain insulin receptors was shown to increase hepatic STAT-3 phosphorylation, resulting in inhibition of PEPCK and G6Pase transcription in the mouse (12). In the present study, insulin increased phosphorylation of STAT-3 in the liver in a dose dependent fashion, but the time course of the activation is not known. We cannot say whether insulin action in the brain was partially responsible for the observed changes in PEPCK since the insulin levels at the liver itself rose markedly. Nevertheless, using the same logic as noted above, it is unlikely that a STAT-3 mediated change in the activity of the gluconeogenic enzymes had any effect on GNG flux to G6P.

In summary, this study demonstrates that a large, but still physiologic rise in plasma insulin can reduce GNG flux to G6P acutely in the conscious dog. However, this effect required a near maximal physiological level of the hormone and was modest compared to the effect on glycogenolysis, even in the 60-h fasted animal. The decrease in GNG flux occurred within 30 min, and was associated with marked inhibition of hepatic fat oxidation, increases in hepatic F2,6P2 level and pyruvate kinase activity, and reductions in hepatic lactate, glycerol and amino acid extraction. No further diminution in GNG
flux 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 gluconeogenic regulation in the dog.

ACKNOWLEDGEMENTS

This research was supported in part by National Institutes of Health grant R37-DK-18243 and the Diabetes Research and Training Center Grant SP-60-AM20593. DSE was supported by the Vanderbilt Molecular Endocrinology Training Program 5T32-DK-07563-12. ADC was supported by the Jacquelyn A. Turner and Dr. Dorothy J. Turner Chair in Diabetes Research.

We wish to thank Dr. Masakazu Shiota, Dr. Rob Hall, Jon Hastings, Angelina Penaloza, Wanda Snead, Patrick Donahue, and Suzan Vaughan (Vanderbilt University) for their excellent technical support. We are grateful to Dr. Alex Lange (University of Minnesota) for providing reagents and expertise.
REFERENCES

Table 1. Arterial plasma non-esterified fatty acid, blood glycerol and β-hydroxybutyrate levels (µmol/l) and net hepatic balances (µmol/kg/min) in 60-h-fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min). Somatostatin and portal insulin and glucagon were infused at 0 min to control hormone levels and glucose was infused to maintain euglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Experimental Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 30</td>
<td>60</td>
</tr>
<tr>
<td><strong>Plasma NEFA Level (µmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>863 ± 110</td>
<td>613 ± 121†</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>813 ± 134</td>
<td>345 ± 66*†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>1151 ± 54*</td>
<td>287 ± 61*†</td>
</tr>
<tr>
<td><strong>Net Hepatic NEFA Uptake (µmol/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.9 ± 0.5</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>2.2 ± 0.6</td>
<td>0.9 ± 0.3*†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>3.1 ± 0.5</td>
<td>0.6 ± 0.2*†</td>
</tr>
<tr>
<td><strong>Plasma Glycerol Level (µmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68 ± 13</td>
<td>58 ± 12</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>88 ± 14</td>
<td>48 ± 11†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>103 ± 8</td>
<td>59 ± 11†</td>
</tr>
<tr>
<td><strong>Net Hepatic Glycerol Uptake (µmol/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.8 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>1.3 ± 0.3</td>
<td>0.6 ± 0.1†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>1.9 ± 0.4</td>
<td>0.9 ± 0.3†</td>
</tr>
<tr>
<td><strong>Plasma β-hydroxybutyrate Level (µmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>72 ± 18</td>
<td>51 ± 16</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>75 ± 28</td>
<td>26 ± 6†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>79 ± 17</td>
<td>21 ± 4†</td>
</tr>
<tr>
<td><strong>Net Hepatic β-hydroxybutyrate Output (µmol/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.3 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>1.6 ± 0.8</td>
<td>0.4 ± 0.3*†</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>2.7 ± 0.7</td>
<td>0.4 ± 0.2*†</td>
</tr>
</tbody>
</table>

Mean ± SEM; n=5, 5 & 6 in the Control, 4x and 16x groups, respectively; *P<0.05 vs. Control group; †P<0.05 vs. basal period.

Table 2. Arterial blood lactate, alanine and gluconeogenic amino acid (alanine, serine, glycine, threonine, glutamate and glutamine) levels (µmol/l), net hepatic fractional extraction and uptake rates (µmol/kg/min), and GNG precursor uptake (mg/kg/min) in 60-h-fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min). Somatostatin and portal insulin and glucagon were infused at 0 min to control hormone levels and glucose was infused to maintain euglycemia.
### Effects of insulin on hepatic gluconeogenesis

#### Basal Experimental Period (min)

<table>
<thead>
<tr>
<th>Blood Lactate Level (µmol/l)</th>
<th>Control</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>409 ± 103</td>
<td>488 ± 122</td>
<td>425 ± 126</td>
<td>429 ± 134</td>
<td>422 ± 121</td>
<td>399 ± 96</td>
<td>352 ± 71</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>474 ± 121</td>
<td>447 ± 82</td>
<td>539 ± 93</td>
<td>480 ± 107</td>
<td>483 ± 122</td>
<td>545 ± 124</td>
<td>555 ± 107</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>393 ± 49</td>
<td>513 ± 59</td>
<td>776 ± 107*†</td>
<td>849 ± 180*†</td>
<td>744 ± 127†</td>
<td>741 ± 85†</td>
<td>705 ± 49*†</td>
</tr>
<tr>
<td>Net Hepatic Lactate Fractional Extraction</td>
<td>Control</td>
<td>0.49 ± 0.06</td>
<td>0.50 ± 0.08</td>
<td>0.50 ± 0.07</td>
<td>0.56 ± 0.10</td>
<td>0.56 ± 0.05</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>0.47 ± 0.10</td>
<td>0.28 ± 0.16*</td>
<td>0.15 ± 0.12*†</td>
<td>0.27 ± 0.06*</td>
<td>0.30 ± 0.06*</td>
<td>0.32 ± 0.06*</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>0.59 ± 0.04</td>
<td>0.28 ± 0.13*†</td>
<td>0.24 ± 0.12*†</td>
<td>0.22 ± 0.03*†</td>
<td>0.29 ± 0.03*†</td>
<td>0.27 ± 0.04*†</td>
</tr>
<tr>
<td>Net Hepatic Lactate Uptake (µmol/kg/min)</td>
<td>Control</td>
<td>7.5 ± 0.9</td>
<td>7.2 ± 1.0</td>
<td>6.3 ± 0.4</td>
<td>6.7 ± 0.7</td>
<td>6.7 ± 0.6</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>6.3 ± 1.4</td>
<td>3.3 ± 1.8</td>
<td>1.6 ± 1.4*†</td>
<td>3.4 ± 0.8</td>
<td>3.8 ± 0.3</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>8.0 ± 1.7</td>
<td>3.6 ± 1.8*†</td>
<td>3.8 ± 1.6†</td>
<td>5.0 ± 1.7</td>
<td>6.2 ± 1.9</td>
<td>5.6 ± 1.4</td>
</tr>
<tr>
<td>Blood Alanine Level (µmol/l)</td>
<td>Control</td>
<td>255 ± 33</td>
<td>256 ± 31</td>
<td>243 ± 36</td>
<td>225 ± 39</td>
<td>219 ± 26</td>
<td>228 ± 22</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>275 ± 44</td>
<td>262 ± 26</td>
<td>248 ± 16</td>
<td>203 ± 17†</td>
<td>175 ± 18†</td>
<td>179 ± 22†</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>266 ± 16</td>
<td>232 ± 14†</td>
<td>231 ± 12†</td>
<td>192 ± 18†</td>
<td>167 ± 16†</td>
<td>141 ± 13*†</td>
</tr>
<tr>
<td>Net Hepatic Alanine Fractional Extraction</td>
<td>Control</td>
<td>0.24 ± 0.04</td>
<td>0.24 ± 0.03</td>
<td>0.32 ± 0.03</td>
<td>0.37 ± 0.02†</td>
<td>0.37 ± 0.03†</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>0.28 ± 0.04</td>
<td>0.34 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>0.45 ± 0.02†</td>
<td>0.46 ± 0.02*†</td>
<td>0.42 ± 0.02*†</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>0.33 ± 0.03</td>
<td>0.34 ± 0.04</td>
<td>0.41 ± 0.04</td>
<td>0.39 ± 0.04</td>
<td>0.46 ± 0.02†</td>
<td>0.45 ± 0.03*†</td>
</tr>
<tr>
<td>Net Hepatic Alanine Uptake (µmol/kg/min)</td>
<td>Control</td>
<td>2.6 ± 0.07</td>
<td>2.2 ± 0.5</td>
<td>2.6 ± 0.4</td>
<td>2.7 ± 0.4</td>
<td>2.4 ± 0.1</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>2.6 ± 0.7</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>3.0 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Blood GNG Amino Acid Level (µmol/l)</td>
<td>Control</td>
<td>1884 ± 161</td>
<td>1810 ± 125</td>
<td>1728 ± 142†</td>
<td>1591 ± 133†</td>
<td>1581 ± 123†</td>
<td>1632 ± 116†</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>1839 ± 87</td>
<td>1754 ± 78</td>
<td>1536 ± 58†</td>
<td>1336 ± 76†</td>
<td>1199 ± 69*†</td>
<td>1199 ± 91*†</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>1575 ± 60</td>
<td>1460 ± 89</td>
<td>1247 ± 79*†</td>
<td>1072 ± 51*†</td>
<td>995 ± 45*†</td>
<td>915 ± 72*†</td>
</tr>
<tr>
<td>Net Hepatic GNG Amino Acid Fractional Extraction</td>
<td>Control</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.15 ± 0.03</td>
<td>0.17 ± 0.03†</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>0.08 ± 0.01</td>
<td>0.15 ± 0.02†</td>
<td>0.17 ± 0.03†</td>
<td>0.20 ± 0.01†</td>
<td>0.15 ± 0.02†</td>
<td>0.20 ± 0.02†</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>0.14 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.12 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Net Hepatic GNG Amino Acid Uptake (µmol/kg/min)</td>
<td>Control</td>
<td>5.5 ± 2.3</td>
<td>5.1 ± 1.7</td>
<td>6.4 ± 1.2</td>
<td>6.3 ± 1.2</td>
<td>7.2 ± 1.5</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>4.3 ± 1.0</td>
<td>6.2 ± 0.5</td>
<td>6.3 ± 0.9</td>
<td>6.6 ± 0.3</td>
<td>4.9 ± 1.0</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>5.6 ± 1.0</td>
<td>4.9 ± 1.0</td>
<td>3.5 ± 0.4</td>
<td>3.2 ± 0.4*</td>
<td>3.8 ± 0.4*</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>GNG Precursor Uptake in Glucose Equivalents (mg/kg/min)</td>
<td>Control</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4x Basal Ins</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>16x Basal Ins</td>
<td>1.5 ± 0.3</td>
<td>0.9 ± 0.2†</td>
<td>0.8 ± 0.1†</td>
<td>0.9 ± 0.2†</td>
<td>1.0 ± 0.2†</td>
<td>0.9 ± 0.1†</td>
</tr>
</tbody>
</table>

Mean ± SEM; n=5, 5 & 6 in the Control, 4x and 16x groups, respectively; *P<0.05 vs. Control group; †P<0.05 vs. basal period.
Table 3. Glucose infusion rate and non-hepatic glucose uptake in 60-h-fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min). Somatostatin and portal insulin and glucagon were infused at 0 min to control hormone levels and glucose was infused to maintain euglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Experimental Period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td><strong>Glucose Infusion Rate (mg/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>0.0 ± 0.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>0.0 ± 0.0</td>
<td>3.2 ± 1.1**†</td>
</tr>
<tr>
<td><strong>Non-Hepatic Glucose Uptake (mg/kg/min)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.8 ± 0.3</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>4x Basal Ins</td>
<td>1.6 ± 0.2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>16x Basal Ins</td>
<td>2.0 ± 0.3</td>
<td>4.5 ± 1.3**†</td>
</tr>
</tbody>
</table>

Mean ± SEM; n=5, 5 & 6 in the Control, 4x and 16x groups, respectively; *P<0.05 vs. Control group; †P<0.05 vs. basal period.

Table 4. Hepatic fructose-2,6-bisphosphate levels and pyruvate kinase activities at the end of the study in 60-h-fasted conscious dogs. Somatostatin and portal insulin and glucagon were infused at 0 min to control hormone levels and glucose was infused to maintain euglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>4x Group</th>
<th>16x Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose-2,6-bisphosphate (nmol/g)</td>
<td>4.6±1.1</td>
<td>11.6±1.7*</td>
<td>26.9±3.3*</td>
</tr>
<tr>
<td>Pyruvate kinase activity (U/mg protein)</td>
<td>0.46±0.06</td>
<td>0.70±0.09*</td>
<td>0.82±0.08*</td>
</tr>
</tbody>
</table>

Mean ± SEM; n=5, 5 & 6 in the Control, 4x and 16x groups, respectively; *P<0.05 vs. Control group.
FIGURE LEGENDS

Fig. 1. Arterial and hepatic sinusoidal plasma insulin and glucagon in 60-h fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min) (mean ± SEM; n=5, 5 and 6 in control, 4x and 16x groups, respectively; *P<0.05 vs. control group; †P<0.05 vs. basal period).

Fig. 2. Arterial plasma glucose level and net hepatic balance in 60-h fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min) (mean ± SEM; n=5, 5 and 6 in control, 4x and 16x groups, respectively; *P<0.05 vs. control group; †P<0.05 vs. basal period).

Fig. 3. Change from basal hepatic gluconeogenic flux to G6P and net hepatic glycogenolytic flux in 60-h fasted conscious dogs during the basal (-40 to 0 min) and experimental periods (0-300 min) (mean ± SEM; n=5, 5 and 6 in control, 4x and 16x groups, respectively; *P<0.05 vs. control group; †P<0.05 vs. basal period). Basal rates of GNG flux were 1.40±0.26, 1.14±0.16 and 1.56±0.26 mg/kg/min, respectively.

Fig. 4. Analysis of liver taken from 60-h fasted conscious dogs following 300 min of treatment. Insulin increased the phosphorylation of Akt (A), GSK-3α and GSK-3β (B) and FOXO-1 (C), which translocated out of the nucleus. G6Pase mRNA expression was reduced by insulin (D) and GK mRNA (E) and protein expression (F) increased. PEPCK mRNA (G) and protein expression (H) decreased. The increase in phosphorylation of STAT3 (I) relative to total STAT3 was insulin dose dependent. Each graph represents mean ± SEM; n=5, 5 and 6 in the control, 4x and 16x groups, respectively; three representative blots are shown for each; *P<0.05 vs. control group; †P<0.05 vs. 4x group.
Effects of insulin on hepatic gluconeogenesis

Figure 1

Figure 2
Effects of insulin on hepatic gluconeogenesis

Figure 3

Hepatic Gluconeogenic Flux to G6P Change from Basal (mg/kg/min)

Breakdown

Net Hepatic Glycogenolysis (mg/kg/min)

Synthesis

TIME (Min)

Control
4x Basal Insulin
16x Basal Insulin
Effects of insulin on hepatic gluconeogenesis

Figure 4

A

P-Ser473 Akt
Total Akt

Relative Akt protein

Control 4X Basal INS 16X Basal INS

B

P-Ser21 GS3
P-Ser5 GS3

Relative GS3 protein

Control 4X Basal INS 16X Basal INS

C

P-Ser286 FOXO1
Nuclear FOXO1

Relative FOXO1 protein

Control 4X Basal INS 16X Basal INS

D

Relative G6Pase mRNA

Control 4X Basal INS 16X Basal INS

E

Relative G6Pase mRNA

Control 4X Basal INS 16X Basal INS

F

Relative G6 protein

G

Relative G6Pase mRNA

Control 4X Basal INS 16X Basal INS

H

Relative PEPCK mRNA

Control 4X Basal INS 16X Basal INS

I

Relative PEPCK protein

Control 4X Basal INS 16X Basal INS

J

Relative STAT3 protein

Control 4X Basal INS 16X Basal INS