Interaction of equal increments in arterial and portal vein insulin on hepatic glucose production in the dog

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Sindelar, Dana K., Chang A. Chu, Doss W. Neal, and Alan D. Cherrington. Interaction of equal increments in arterial and portal vein insulin on hepatic glucose production in the dog. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E972–E980, 1997.—We have previously shown that a selective increase of 84 pmol/l in either arterial or portal vein insulin (independent of a change in insulin in the other vessel) can suppress tracer-determined glucose production (TDGP) and net hepatic glucose output (NHGO) by ~50%. In the present study we investigated the interaction between equal increments in arterial and portal vein insulin in the suppression of TDGP and NHGO. Isotopic ([3-3H]glucose) and arteriovenous difference methods were used in conscious overnight fasted dogs. A pancreatic clamp was used to control the endocrine pancreas. A 40-min basal period was followed by a 180-min test period, during which arterial and portal vein insulin levels were simultaneously and equally increased 102 pmol/l. Hepatic sinusoidal glucagon levels remained unchanged, and euglycemia was maintained by peripheral glucose infusion. TDGP was suppressed ~60% by the last 30 min of the experimental period. In contrast, NHGO was suppressed 100% by that time. Coincidentally, hepatic glucose uptake (net hepatic [3H]glucose balance) increased significantly (~4 µmol · kg⁻¹ · min⁻¹). The effects of simultaneous equal increases in peripheral and portal venous insulin were not additive in the suppression of TDGP. However, they were additive in decreasing NHGO as a result of an increase in the uptake of glucose by the liver.

MATERIALS AND METHODS

Animal care and surgical procedures. Experiments were conducted on 11 conscious mongrel dogs (18–30 kg) of either sex that had been fed a meat and chow diet (34% protein, 46% carbohydrate, 14.5% fat, and 5.5% fiber based on dry weight; Kal Kan (Vernon, CA) beef dinner and Purina Lab Canine Diet No. 5006) once daily. 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.

Each dog underwent a laparotomy performed with the animal under general anesthesia (15 mg/kg pentothal sodium, presurgery, and 1% isoflurane inhalation anesthetic during surgery) 2 wk before the experiment. With the use of previously described sterile techniques (2), Silastic catheters (0.03-in. ID; Dow Corning, Midland, MI) were placed into a splenic and jejunal vein for intraportal infusions as required. Catheters (0.04-in. ID) for blood sampling were placed in the left common hepatic vein, the hepatic portal vein, and the femoral artery, as described previously (10). All catheters were filled with saline containing heparin (200 U/ml; Abbott Laboratories, North Chicago, IL), and their free ends were knotted before closure of the skin. Doppler flow probes (Instrument Development Laboratories, Baylor College of Medicine, Houston, TX) were placed around the hepatic artery and portal vein to determine hepatic blood flow, as previously described (24). The Doppler leads, along with the catheters, were placed in a subcutaneous pocket before closure of the abdominal skin. The positions of the catheter tips were confirmed with autopsy.

Only dogs that had a leukocyte count <18,000/mm³, a hematocrit >35%, normal stools, and had consumed their daily food ration were used for a study. On the day of the experiment, after an 18-h fast, the catheters and flow probe leads were exteriorized, with the animal under local anesthesia (2% lidocaine; Astra Pharmaceutical, Worcester, MA). The contents of each catheter were aspirated, and the catheters were aborted.
were flushed with saline. The intraportal catheters (splenic and jejunal) were used for the infusion of insulin and glucagon (Lilly, Indianapolis, IN). Angiocaths (Deseret Medical, Becton-Dickinson, Sandy, UT) were inserted percutaneously into the left cephalic vein for [3-3H]glucose (NEN, Boston, MA) plus indocyanine green (Becton-Dickinson, Cockeysville, MD) infusion and into a saphenous vein for somatostatin (Bachem, Torrance, CA) plus insulin infusion. An angiocath was inserted into the right cephalic vein for peripheral glucose infusion. Each animal was allowed to rest quietly in a Pavlov harness for 30 min before the experiment was begun.

Experimental procedure. Each experiment consisted of a tracer and dye equilibration period (−140 to −40 min), a basal period (−40 to 0 min), and an experimental period (0 to 180 min). At −140 min, a priming dose of [3-3H]glucose (25 µCi) was given and a continual infusion of [3-3H]glucose (0.21 µCi/min) was begun to allow assessment of HGP. Constant infusions of indocyanine green (0.07 mg/min) and somatostatin (0.8 µg·kg\(^{-1}·\text{min}^{-1}\)) were started simultaneously (t = −140 min) via a leg vein to measure hepatic blood flow and to inhibit the endogenous secretion of insulin and glucagon, respectively. A constant intraportal infusion of glucagon (0.5 ng·kg\(^{-1}·\text{min}^{-1}\)) was given to replace endogenous glucagon secretion. A constant infusion of insulin was given via a peripheral vein, and a variable insulin infusion was given via the portal infusion catheters to replace endogenous insulin secretion. The rate of the portal insulin infusion was adjusted to maintain preexisting plasma glucose levels. Once the plasma glucose level had been stabilized at a euglycemic value for 30 min, the basal sampling period was begun. The rate of [3H]glucose infusion during the experimental period in the test group was adjusted to clamp the arterial plasma glucose level had been stabilized at a euglycemic value for 30 min, the basal sampling period was begun. The rate of [3H]glucose infusion during the experimental period in the test group was adjusted to clamp the arterial plasma glucose level at the value that preexisted during the basal period (140 to 180 min). At 0 min, the portal insulin infusion was raised 0.54 pmol·kg\(^{-1}·\text{min}^{-1}\) and the peripheral insulin infusion was increased 1.5 pmol·kg\(^{-1}·\text{min}^{-1}\). On the basis of our earlier studies (27, 28), it was calculated that these changes in the insulin infusion rates would increase arterial and portal vein insulin levels ∼84 pmol/l. These rates are in line with those used in our earlier studies (27, 28), although, in the previous paper, they were mistakenly reported to be 102 greater than they actually were. Euglycemia was maintained during the experimental period using a variable glucose infusion given through a peripheral vein. In this way we were able to create simultaneous and equal increases in both the arterial and portal vein insulin concentrations.

Protocol 1: Combined simultaneous equal increase in peripheral and portal vein insulin group. During the basal period, the portal insulin infusion rate averaged 1.29 pmol·kg\(^{-1}·\text{min}^{-1}\), whereas the peripheral insulin infusion rate was 0.48 pmol·kg\(^{-1}·\text{min}^{-1}\). At 0 min, the portal insulin infusion was raised 0.54 pmol·kg\(^{-1}·\text{min}^{-1}\) and the peripheral insulin infusion was increased 1.5 pmol·kg\(^{-1}·\text{min}^{-1}\). On the basis of our earlier studies (27, 28), it was calculated that these changes in the insulin infusion rates would increase arterial and portal vein insulin levels ∼84 pmol/l. These rates are in line with those used in our earlier studies (27, 28), although, in the previous paper, they were mistakenly reported to be 102 greater than they actually were. Euglycemia was maintained during the experimental period using a variable glucose infusion given through a peripheral vein. In this way we were able to create simultaneous and equal increases in both the arterial and portal vein insulin concentrations.

Protocol 2: Control group. Control studies (Cont, n = 5) were carried out as described in protocol 1 except that, on completion of the basal period, no changes were made in the site or rate of insulin delivery. The portal insulin infusion averaged 0.48 pmol·kg\(^{-1}·\text{min}^{-1}\), and the peripheral insulin infusion was 0.48 pmol·kg\(^{-1}·\text{min}^{-1}\). Glucose infusion was not required. In this way we controlled for changes in HGP, which might occur over time even in the absence of a change in insulin. The data from this group have been presented elsewhere (27).

Arterial blood samples were taken every 10 min during the basal period and every 15 min during the experimental period. Portal and hepatic blood samples were drawn every 20 min during the basal period, 15 and 30 min after the initiation of the experimental period, and every 30 min thereafter. The arterial plasma glucose level was monitored every 5 min during the experimental period to assess glycemia. The total volume of blood withdrawn did not exceed 20% of the animal’s blood volume, and two volumes of saline were given for each volume of blood withdrawn. No significant decreases in hematocrit occurred with this procedure (<5%).

The arterial and portal blood samples were collected simultaneously ∼30 s before collection of the hepatic venous sample in an attempt to compensate for transit time of glucose through the liver (14) and thus allow the most accurate estimates of net hepatic balance to be obtained.

Analytic procedures. Blood samples were processed for the later determination of acetocetate, β-hydroxybutyrate, glycerol, and lactate and the gluconeogenic amino acids alanine, glutamine, glutamate, glycine, serine, and threonine.

For the determination of plasma [3H]glucose, 1-ml plasma samples were deproteinized with 5 ml of 0.067 N barium hydroxide and 5 ml of 0.067 N zinc sulfate (Sigma Chemical). A 5-ml aliquot of the supernatant of the euglycemic value for 30 min, the basal sampling period was begun. The rate of [3H]glucose infusion during the experimental period in the test group was adjusted to clamp the arterial plasma glucose specific activity at the value that preexisted during the basal period. This was not done in the control group because of the continued existence of a steady state for glucose.

Arteriovenous difference calculations. The NHGO and the arteriovenous difference calculations.

Tracer calculations. Rates of tracer-determined total HGP (R\(_{\text{a}}\)) and tracer-determined glucose utilization were measured using a primed, continual infusion of [3-3H]glucose. Data calculation was carried out using a two-compartment model described by Mari (19) using canine parameters reported by Dobbins et al. (11). TDGP was calculated as the difference between R\(_g\) and the exogenous glucose infusion rate.

Arteriovenous difference calculations. The NHGO and the net hepatic balance of gluconeogenic substrates were calculated using the formula \[
\text{NHGO} = (H - (0.28A + 0.72P)) \times HF,
\]
where H, A, and P are the substrate concentrations in the hepatic vein,
RESULTS

Effects of a combined increase in peripheral and portal vein insulin. When simultaneous increments in arterial and portal insulin were brought about, the arterial insulin level rose from 66 ± 9 (basal) to 168 ± 10.2 pmol/l by the last 30 min of the study (Fig. 1) and the portal insulin level increased similarly (210 ± 47 to 312 ± 45 pmol/l). Sinusoidal insulin therefore increased from ~170 (basal) to 272 pmol/l (last 30 min). The hepatic sinusoidal glucagon level fell slightly (~10%), whereas the arterial plasma glucose concentration and plasma glucose specific activity remained unaltered (Table 1). TDGP fell from 12.4 ± 0.7 to 7.9 ± 0.9 µmol·kg⁻¹·min⁻¹ by 30 min into the experimental period and was suppressed to 5.3 ± 0.8 µmol·kg⁻¹·min⁻¹ by the last 30 min of the study (Fig. 2, P < 0.05). NHGO fell from 9.8 ± 2.2 to 4.0 ± 1.2 by 30 min and to 0.0 ± 1.6 µmol·kg⁻¹·min⁻¹ by the last 30 min of the study (Fig. 2, P < 0.05). The rise in insulin increased whole body glucose utilization by more than twofold (Table 1, P < 0.05). Interestingly, total hepatic glucose uptake (calculated from net hepatic [3-3H]glucose balance) increased significantly from 1.2 ± 0.1 (basal) to 4.3 ± 0.2 µmol·kg⁻¹·min⁻¹ by 1 h into the experimental period (P < 0.05) and remained elevated for the rest of the study (4.2 ± 0.5 µmol·kg⁻¹·min⁻¹, last 30 min, Fig. 3). Arterial blood lactate levels (Table 2) did not change during the course of the study. Net hepatic lactate...
Production (Fig. 4) rose significantly (P < 0.05), reaching 5.1 ± 2.4 µmol·kg⁻¹·min⁻¹ by 90 min, and then fell to 1.6 ± 1.6 µmol·kg⁻¹·min⁻¹ by the end of the study. The arterial blood glycerol level dropped ~35% (Table 2), whereas arterial blood gluconeogenic amino acid levels dropped ~20% (Table 4). Net hepatic uptake of glycerol declined slightly from 1.1 ± 0.4 (basal) to 0.9 ± 0.2 µmol·kg⁻¹·min⁻¹ (Table 3, P < 0.05), whereas the net hepatic uptake of the gluconeogenic amino acids did not change (4.5 ± 0.7, basal, to 4.4 ± 1.0 µmol·kg⁻¹·min⁻¹, last 30 min, Table 3). Total net hepatic gluconeogenic precursor uptake (including changes in net hepatic lactate uptake when such occurred) declined modestly from 7.6 ± 1.7 (basal) to 5.7 ± 1.2 µmol·kg⁻¹·min⁻¹ (last 30 min, P < 0.05). This fall in hepatic gluconeogenic precursor uptake (1.9 µmol·kg⁻¹·min⁻¹) could account for a drop of no more than ~1.0 µmol·kg⁻¹·min⁻¹ in glucose production.

The arterial plasma NEFA level dropped significantly from 560 ± 60 (basal) to 248 ± 31 µmol·l⁻¹ (P < 0.05) by 1 h and remained suppressed thereafter (Table 2). Net hepatic NEFA uptake showed a similar pattern of suppression (Fig. 5). Arterial acetacetate and β-hydroxybutyrate levels fell ~60% and ~30%, respectively (Table 2), as the result of a fall in net hepatic ketone production from 1.4 ± 0.2 (basal) to 0.8 ± 0.2 µmol·kg⁻¹·min⁻¹ (last 30 min, Table 3).

Control group. Neither arterial, portal, nor hepatic sinusoidal insulin levels changed over the course of the control experiments (Fig. 1). Once again the hepatic sinusoidal glucagon level fell slightly (~10%), whereas both the arterial plasma glucose concentration and the arterial plasma glucose specific activity remained unchanged (Table 1). TDGP declined from 13.7 ± 0.8 to 11.0 ± 0.6 µmol·kg⁻¹·min⁻¹ by the last 30 min of the experimental period (P < 0.05, Fig. 2). NHGO showed a similar decline (P < 0.05) from 10.5 ± 0.7 (basal) to 8.4 ± 1.1 µmol·kg⁻¹·min⁻¹ (Fig. 2), whereas whole body glucose utilization declined minimally over the course of the experiment (Table 1). Hepatic glucose uptake (net hepatic [3-⁴H]glucose balance) did not change (2.5 ± 0.3, basal, to 2.4 ± 0.6 µmol·kg⁻¹·min⁻¹, last 30 min, Fig. 3).

The arterial blood lactate level (Table 2) did not change even though net hepatic lactate output dropped modestly (8.0 ± 2.9, basal, to 3.9 ± 1.7 µmol·kg⁻¹·min⁻¹, last 30 min, Fig. 4). Neither the arterial blood gluconeogenic amino acid levels (Table 4) nor the arterial blood glycerol level (Table 3) changed significantly. The net hepatic uptake of gluconeogenic amino acids increased slightly (4.3 ± 0.2, basal, to 5.7 ± 1.3 µmol·kg⁻¹·min⁻¹, last 30 min), whereas the net hepatic uptake of glycerol did not change (0.9 ± 0.1, basal, to 0.9 ± 0.3 µmol·kg⁻¹·min⁻¹, last 30 min, Table 3). As a result, total net gluconeogenic precursor uptake by the liver (including changes in net hepatic lactate uptake when such occurred) increased slightly from 5.2 ± 0.3 (basal) to 6.9 ± 1.4 µmol·kg⁻¹·min⁻¹ (last 30 min, P = 0.1) in the control study.

Arterial plasma NEFA levels (640 ± 120, basal, to 670 ± 94 µmol·l⁻¹, last 30 min, Table 2), net hepatic NEFA uptake (2.2 ± 0.5, basal, to 2.4 ± 0.4 µmol·kg⁻¹·min⁻¹, last 30 min, Fig. 5), arterial acetacetate and β-hydroxybutyrate levels (Table 2), and net hepatic ketone production (1.0 ± 0.2, basal, to 1.2 ± 0.2 µmol·kg⁻¹·min⁻¹, last 30 min, Table 3) did not change significantly in the control group.

**DISCUSSION**

The combination of equal increases of 102 pmol·l⁻¹ in peripheral and portal vein insulin suppressed TDGP...
60% and NHGO 100%. The difference in the two estimates of suppression can be explained by the ability of the combination of portal and peripheral insulin to increase hepatic glucose uptake, which would only be apparent in NHGO. Such an increase did not occur in response to a similar rise in arterial or portal insulin alone (27). Hepatic glucose uptake must therefore have increased either because the liver sinusoidal insulin level was higher (60%) when the increments were brought about together or because the combination of the rise in portal vein and peripheral insulin has a unique effect. In response to the simultaneous and equal increases in arterial and portal vein insulin, TDGP decreased 4.4 µmol·kg\(^{-1}\)·min\(^{-1}\) over and above the fall evident in the control protocol. In our previous studies, selective and independent 84-pmol/l increases in arterial and portal vein insulin suppressed TDGP by 5.2 and 4.4 µmol·kg\(^{-1}\)·min\(^{-1}\) (by 180 min), respectively, relative to a control study. Obviously, the effects of combined increases in peripheral and portal vein insulin were not additive in suppression of TDGP (relative to the control study). When portal insulin was selectively raised in our previous study, glycogenolysis was completely inhibited. During the selective increase in peripheral insulin, 1.8 µmol·kg\(^{-1}\)·min\(^{-1}\) of the decrease in NHGO was due to an inhibition of glycogenolysis (resulting from the increase in hepatic sinusoidal insulin that resulted from the rise in arterial insulin) and 1.8 µmol·kg\(^{-1}\)·min\(^{-1}\) was due to the redirection of glycogenolytically derived carbon to lactate. In the present experiments, neither of the latter would be expected to occur in response to the increment in peripheral insulin, since the increase in hepatic sinusoidal insulin resulting from the concurrent increase in peripheral and portal insulin was greater (60%) than the increase previously experienced and would have inhibited glycogenolysis completely. Thus, in the Combo group, one would have expected an inhibition of glycogenolysis (−4.4 µmol·kg\(^{-1}\)·min\(^{-1}\)) along with a fall in net hepatic gluconeogenesis (1.6 µmol·kg\(^{-1}\)·min\(^{-1}\)), which would have resulted from the rise in portal insulin. If the drop in TDGP (2.7 µmol·kg\(^{-1}\)·min\(^{-1}\)) in the Cont group is taken into account, a fall of 8.7 µmol·kg\(^{-1}\)·min\(^{-1}\) from baseline would be predicted in the Combo group. The observed TDGP fall in the Combo group was 7.1 µmol·kg\(^{-1}\)·min\(^{-1}\), a value very close to that predicted.

The inability of small increases in insulin to fully suppress TDGP is not an unexpected result. In humans, Katz et al. (16) and Hother-Nielsen et al. (15) found the 50% effective dose for the suppression of HGP by arterial insulin to be −95–165 pmol/l. Bevilacqua et
al. (4) raised arterial insulin 360 pmol/l in humans during euglycemia and found that HGP was suppressed from $66 \pm 7$ to $15 \pm 8$ mg·m$^{-2}$·min$^{-1}$ by 120 min. In all of the above studies, however, glucagon was either not measured or fell, and this change most likely contributed to the decrease in glucose production. Boden et al. (5) found that an increase in arterial insulin from 30 to 420 pmol/l suppressed HGP from 12.5 to 1.6 µmol·kg$^{-1}$·min$^{-1}$ even when the glucagon concentration did not change. In agreement with our data, these previous studies demonstrated that glucose production by the liver, as measured by tracer methodology, is not completely inhibited, even by relatively high insulin concentrations.

NHGO in the Combo group was fully suppressed (9.8 to 0.0 µmol·kg$^{-1}$·min$^{-1}$) by insulin infusion. Because NHGO represents the net movement of glucose into and out of the liver, and since TDGP fell to only 5.3 µmol·kg$^{-1}$·min$^{-1}$, the suppression of NHGO must have resulted in part from an increase in hepatic glucose uptake. The difference between TDGP (5.3 µmol·kg$^{-1}$·min$^{-1}$) and NHGO (0 µmol·kg$^{-1}$·min$^{-1}$) in the last 30 min of the study provides an estimate of the net amount of glucose taken up by the liver (5.3 µmol·kg$^{-1}$·min$^{-1}$). To the extent that the kidneys are responsible for a portion of TDGP, this would be an overestimate. In our earlier study (20), the kidney produced $\sim 2.0$ µmol·kg$^{-1}$·min$^{-1}$ of glucose after an overnight fast in the conscious dog. A study by Cersosimo et al. (7) demonstrated that a selective increase of arterial plasma NEFA could decrease renal glucose release by 73%. Therefore, because the rise in arterial insulin in the Combo group was 102 pmol/l, renal glucose release would be expected to be virtually zero and thus TDGP would be a reflection of glucose release by the liver. The net hepatic [3-3H]glucose balance data confirm that hepatic glucose uptake was increased during hyperinsulinemia (1.2 basal to 4.2 µmol·kg$^{-1}$·min$^{-1}$ by the end of the experiment). Both estimates of hepatic glucose uptake suggest that it was 4–5 µmol·kg$^{-1}$·min$^{-1}$ during the increase in insulin.

If glycogenolysis is shut off by the rise in portal insulin as suspected, the carbon produced by the liver as glucose (5.3 µmol·kg$^{-1}$·min$^{-1}$) and lactate (0.8 µmol·kg$^{-1}$·min$^{-1}$ glucose equivalents), as well as the amount theoretically oxidized within the liver (–2.0 µmol·kg$^{-1}$·min$^{-1}$), must be derived from the gluconeogenic precursors being taken up (2.9 µmol·kg$^{-1}$·min$^{-1}$ glucose equivalents) and exogenous glucose extracted.
Table 3. Net hepatic uptake of glycerol and net hepatic production of β-OHB and AcAc in 18-h fasted conscious dogs studied during a basal period and with or without a simultaneous equal increase in peripheral and portal vein insulin level

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
<th>Experimental Period, min</th>
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<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Net hepatic glyceral uptake, µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combo</td>
<td>1.2±0.3</td>
<td>0.7±0.1*</td>
</tr>
<tr>
<td>Cont</td>
<td>0.9±0.1</td>
<td>0.6±0.1</td>
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<tr>
<td>Net hepatic β-OHB production, µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combo</td>
<td>0.8±0.1</td>
<td>0.6±0.1*</td>
</tr>
<tr>
<td>Cont</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
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<tr>
<td>Net hepatic AcAc production, µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combo</td>
<td>0.6±0.2</td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>0.5±0.3</td>
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Values are group means ± SE; 5 dogs in each group. Basal period value is the average of the -40, -20, and 0 min time points. Significant differences (P < 0.05) from * basal values are noted.

Table 4. Arterial blood concentrations and net hepatic uptake rates of the gluconeogenic amino acids in 18-h fasted conscious dogs studied during a basal period and with or without a simultaneous equal increase in the peripheral and portal vein insulin level

<table>
<thead>
<tr>
<th></th>
<th>Alaine</th>
<th>Glutamine</th>
<th>Glutamate</th>
<th>Glycine</th>
<th>Serine</th>
<th>Threonine</th>
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<tr>
<td></td>
<td>Basal</td>
<td>Exp</td>
<td>Basal</td>
<td>Exp</td>
<td>Basal</td>
<td>Exp</td>
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<tr>
<td>Arterial blood</td>
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<tr>
<td>level, µmol/l</td>
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<tr>
<td>Combo</td>
<td>390±40</td>
<td>325±35</td>
<td>894±65</td>
<td>810±61</td>
<td>108±8</td>
<td>95±6</td>
</tr>
<tr>
<td></td>
<td>Cont</td>
<td>520±60</td>
<td>488±60</td>
<td>734±130</td>
<td>747±134</td>
<td>86±20</td>
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<td></td>
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<tr>
<td>Net hepatic</td>
<td></td>
<td></td>
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<tr>
<td>uptake, µmol·kg⁻¹·min⁻¹</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combo</td>
<td>2.7±0.6</td>
<td>2.7±0.6</td>
<td>-0.5±0.8</td>
<td>-0.1±0.5</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td></td>
<td>Cont</td>
<td>2.7±0.3</td>
<td>3.3±0.5</td>
<td>-0.6±0.4</td>
<td>-0.7±0.4</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Arterial blood concentrations are group means ± SE for 6 and 5 dogs in Combo and Cont groups, respectively. Net hepatic uptake data are means ± SE for 5 dogs each group. Basal period value is average of the -40, -20, and 0 min time points, and the experimental period (Exp) value is average of the 150 and 180 time points. (A negative value represents a mean production for the group.)

by the liver. This suggests that glucose uptake by the liver was -5.2 µmol·kg⁻¹·min⁻¹, again in tune with the above estimates. To the extent that glycogenolysis continued, the need for hepatic glucose uptake would be reduced. Regardless of its absolute value, it is clear that, when insulin was raised in the peripheral circulation and portal vein concurrently, hepatic glucose uptake occurred. Thus the effects of equal 102-pmol/l increases in peripheral and portal vein insulin brought about simultaneously are additive in suppression of NHGO in that they cause a significant movement of glucose into the hepatocyte, which neither causes alone. As noted previously, the latter could be explained by an interaction of the direct and indirect actions of insulin on the liver or by the fact that the simultaneous increase in arterial and portal insulin created a greater rise in the liver sinusoids than when either was increased alone.

During a selective increase in peripheral insulin, net hepatic lactate output increased significantly, whereas NHGO fell (27). Glycogenolytically derived carbon was redirected through glycolysis to lactate without a net decrease in glycogen breakdown. These changes correlated with a temporal fall in the NEFA level. In fact, when NEFA levels were maintained, the increase in net...
hepatic lactate output did not occur and the fall in NHGO was significantly blunted (28). A selective increase in portal insulin, on the other hand, suppressed glycolysis, failed to alter NEFA levels, and suppressed net hepatic lactate output (27). In the current studies, when portal and peripheral insulin levels were increased simultaneously, NEFA levels again fell. As a result, net hepatic lactate output again increased significantly 60 min into the experiment and was still increased by 1.8 µmol·kg\(^{-1}\)·min\(^{-1}\) at the end of the study. In the control group, net hepatic lactate output decreased progressively, eventually falling by 5.1 µmol·kg\(^{-1}\)·min\(^{-1}\). If the difference between the two groups is converted to an amount of glucose that was broken down to create this amount of lactate, it would equal 3.5 µmol·kg\(^{-1}\)·min\(^{-1}\) of glucose equivalents. Interestingly, the significant increase in lactate output correlated temporally with the increase in glucose uptake by the liver. Thus glycolysis was again increased, even though glycolysis was inhibited, but in this case the glucose carbon being converted to lactate seems to have been derived from the circulation. The exact biochemical mechanism by which the fall in NEFA levels influences the increase in net hepatic lactate output and net hepatic uptake of glucose is unknown. The fall in NEFA may bring about multiple effects, including potential changes in substrate levels and the redox state of the liver. A fall in citrate levels would activate phosphofructokinase, whereas a change in the mitochondrial redox state could activate pyruvate dehydrogenase, both of which could enhance glycolysis and in turn cause lactate dehydrogenase to convert pyruvate to lactate. The difference in activation time of the enzymes may cause the peak in net hepatic lactate output at 1 h and the eventual decrease in lactate output by the end of the experiment.

Other investigators have shown (8,9) that, in euglycemic humans, insulin levels >600 pmol/l can increase splanchnic glucose uptake slightly (3.9 µmol·kg\(^{-1}\)·min\(^{-1}\)). Similarly, in the dog (21) net hepatic glucose uptake was 3.3 µmol·kg\(^{-1}\)·min\(^{-1}\) during euglycemic hyperinsulinemia of 720 pmol/l. No experiments to date have clearly shown that hepatic glucose uptake is increased in response to small changes in insulin (102 pmol/l) under euglycemic conditions. It has been shown, however, that relatively small changes in arterial insulin (144 pmol/l) can result in net hepatic uptake of glucose under hyperglycemic conditions (24). The results from the current study may be interpreted to indicate that peripheral insulin, working through the suppression of NEFA levels, could play a role in regulating the uptake of glucose by the liver. However, the effect can only occur when hepatic sinusoidal insulin has increased enough to suppress glycolysis. The consequences of NEFA suppression under hyperinsulinemic conditions on the movement of glucose into the liver remain to be investigated.

These findings may indicate why other investigators (1,3,6) have concluded that insulin's ability to suppress HGP appears relatively slow (~60 min). Insulin appears to have three different effects on the liver, which occur at different times. One action of insulin is an immediate and direct effect on the liver to suppress glycolysis. We have previously demonstrated that a selective increase of 84 pmol/l in portal vein insulin rapidly (~15 min) and completely suppresses glycolysis (27). A secondary effect appears dependent on an increase in glucose uptake by the liver, which is maximal at 1 h. In a previous study, Pagliassotti et al. (25) showed that it required 45 min for an effect of insulin on net hepatic glucose uptake to become manifest under hyperglycemic conditions. A third effect appears to be a time-dependent suppression of the gluconeogenic precursor supply to the liver. The sum of these three effects is to create a rapid first-phase suppression of HGP in combination with a slower secondary phase. Therefore, the overall response of HGP to insulin appears to be slow (~60 min). It is important to note that the first phase, which represents a suppression of glycolysis, is rapid (~15 min).

In summary, we found that simultaneous equal increases in peripheral and portal vein insulin of 102 pmol/l were not additive in their ability to suppress TDGP. They were, however, additive in their ability to decrease NHGO. The latter occurred because the effects of the changes in insulin were synergistic in increasing the uptake of glucose by the liver. Whether this was due to the greater increase in sinusoidal insulin or the result of a unique response to the combined direct (liver) and indirect (fat) effects of insulin remains to be determined.

The authors thank J. on Hastings, Pam Venson, Wanda Snead, Paul Flakoll, and Annapurna Venkatarkrishnan for excellent technical assistance.

This work was presented in part at the 56th Annual Meeting of the American Diabetes Association, San Francisco, CA, June 8–11, 1996. Present address of D. K. Sindelar: Metabolism (151), Dept. of Veterans Affairs, 1660 South Columbian Way, Seattle, WA 98108–1597.

This research was supported in part by Grants 2R01 DK-18243 and 5P60 DK-2059 from the National Institute of Diabetes and Digestive and Kidney Diseases.

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PORTAL AND PERIPHERAL INSULIN ACTION ON THE LIVER


