Insulin acutely suppresses glucose production by both peripheral and hepatic effects in normal dogs

McCall, Richard H., Stephanie R. Wiesenthal, Z. Qing Shi, Kenneth Polonsky, and Adria Giacca. Insulin acutely suppresses glucose production by both peripheral and hepatic effects in normal dogs. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E346–E356, 1998.—To determine whether the predominant effect of insulin in suppressing tracer-determined glucose production (Rₕ) is hepatic or peripheral, we infused insulin peripherally (PER) and portally (POR) at both low (0.75 pmol·kg⁻¹·min⁻¹) and high physiological rates (2.7 pmol·kg⁻¹·min⁻¹) during euglycemic clamps in normal dogs. We also infused insulin peripherally at one-half these rates (1/2 PER) to match the peripheral insulin levels in POR and thus obtain a selective POR vs. 1/2 PER difference in hepatic insulin levels. At the high-rate insulin infusion, peripheral insulin levels were greater with PER (PER = 22 ± 10 pM, n = 5; POR = 119 ± 5 pM, n = 6; 1/2 PER = 122 ± 5 pM, n = 6). Calculated hepatic insulin levels were greater with POR (POR = 227 ± 13 pM, PER = 206 ± 19 pM, 1/2 PER = 212 ± 8 pM). High-dose PER yielded a greater suppression of Rₕ than POR (79 ± 18 vs. 56 ± 6%; P < .001). Rₕ was only suppressed by 45 ± 6% with 1/2 PER (P < 0.01 vs. POR on 6 paired experiments). Free fatty acid (FFA) was suppressed by 57 ± 8% with PER and only by 33 ± 5 and 37 ± 2% with POR and 1/2 PER, respectively. The low-dose PER and POR yielded an equal Rₕ suppression (PER = 46 ± 9%, POR = 43 ± 4%). Only 1/2 PER was associated with a lower suppression of Rₕ (36 ± 8, P < 0.05 vs. POR). FFA showed similar suppression in all three groups (–25%). Using both insulin infusion rates, the percent Rₕ suppression per unit difference in peripheral insulin was approximately twofold greater than that per unit difference in hepatic insulin. These results suggest that, during euglycemic clamps without somatostatin in normal dogs, Rₕ suppression is mediated by both peripheral and hepatic effects of insulin and that peripheral insulin, at least at high physiological infusion rates, is more potent than hepatic insulin in suppressing Rₕ.

In normal physiology, insulin has a strong inhibitory effect on tracer-determined glucose production (rate of glucose appearance = Rₕ), but the extent to which this represents a direct action of hepatic sinusoidal insulin (i.e., inhibition of glycolysis and/or glucoseogenesis via insulin’s interactions with its hepatocyte receptor) or action of peripheral insulin via its effects on extrahepatic tissues is still unclear. The peripheral action of insulin on Rₕ has been recently linked to insulin-induced inhibition of lipolysis (16, 24, 28).

In obese humans, Prager et al. (22) infused insulin peripherally and, by suppressing endogenous insulin secretion, increased peripheral but not estimated portal insulin levels. They found that Rₜ was significantly suppressed (82%), which is consistent with Rₜ inhibition by peripheral insulin. Ader and Bergman (1), using improved glucose tracer techniques in normal dogs, directly demonstrated that Rₜ suppression was mediated by peripheral insulin. They could not detect any hepatic action of insulin on Rₜ. Sindelar et al. (27) showed that a selective increase in peripheral insulin levels suppressed Rₜ and a selective increase in portal insulin levels also suppressed Rₜ in normal dogs. Thus, although all of the recent studies show that Rₜ is inhibited by peripheral insulin, there is still disagreement as to whether Rₜ can also be inhibited by hepatic insulin. We have shown that insulin-induced inhibition of Rₜ is proportional to peripheral insulin levels in depancreatized dogs (12) and to both hepatic and peripheral insulin levels in normal humans (17). The difference between dogs and humans might relate to the diabetic state, to species specificity, or to the experimental model used to induce portal insulin secretion in humans (tolbutamide infusion). Because the dog is generally considered a good model for the study of glucose metabolism and because we have shown that, in type 1 diabetic individuals, tolbutamide does not affect glucose turnover independent of its insulin-releasing action (17), we hypothesized that similar results would be obtained in normal dogs and humans, and therefore the differences between our results in dogs (12) and humans (17) could be related to the diabetic state of our dogs. To test this hypothesis, we investigated the mechanism of Rₜ inhibition by insulin in normal dogs. In contrast to previous studies (1, 27), we did not use somatostatin to inhibit endogenous insulin secretion for two reasons: first, although under most conditions somatostatin has no significant metabolic effects (6), it may independently affect glucose turnover and lipolysis (3, 13) under some conditions and thus could potentially alter the balance between insulin’s hepatic and peripheral effects; second, we wished to make our results comparable with those obtained in depancreatized dogs and in humans, where somatostatin was not used. However, we determined dog C-peptide levels to obtain a measure of endogenous insulin secretion and its suppression by insulin. The experimental design consisted of the following protocols: 1) portal insulin infusion (POR), 2) equidose peripheral insulin infusion (PER), and 3) one-half dose peripheral insulin infusion (1/2 PER). The peripheral insulin levels were higher with PER than with POR, whereas the calculated hepatic insulin levels were lower. Thus a greater Rₜ suppression with PER than with POR would indicate that Rₜ is suppressed by a peripheral effect of insulin. POR and 1/2 PER only
differed in the hepatic insulin levels, which were greater with POR, whereas the peripheral insulin levels were matched by experimental design. Thus a greater Ra suppression with POR than with 1/2 PER would indicate that Ra is suppressed by a direct hepatic effect of insulin. Our aim was to determine whether peripheral or hepatic insulin levels or both suppress Ra in normal dogs and to quantify their effects. Because the relative contribution of peripheral vs. hepatic effects of insulin may depend on the insulin dose, we studied two insulin concentrations only slightly above the fasting range.

METHODS

Experimental animals and preparation. The studies were performed on seven postabsorptive, normal dogs of either sex. Mongrel dogs weighing 22–34 kg and of at least 1 yr of age underwent vessel cannulation performed under general anesthesia induced with sodium thiopental and maintained with halothane and nitrous oxide and assisted ventilation. A Silastic cannula (0.04-in. internal diameter; Baxter Healthcare, McGaw Park, IL) was inserted into the portal vein through a branch of the splenic vein and advanced until the tip was ~1.0 cm beyond the point of confluence of the splenic vein with the portal vein, i.e., ~5 cm from the branching point of the portal vein into its left and right bifurcations to the liver. The portal cannula served for infusion. We did not have sampling catheters in the portal vein, as we chose not to use the triple-catheter technique (20) for these studies. This was due to the high failure rate of the catheters with this technique, which makes it difficult to carry out at least three paired experiments in the same dog. Three additional Silastic cannulas served for peripheral infusion (one 0.04-in. internal diameter and two 0.03-in. internal diameter catheters). The three cannulas were inserted into the jugular vein and advanced into the superior vena cava. In addition, a Silastic cannula (0.04-in. internal diameter) was inserted into a carotid artery and advanced into the aortic arch. The arterial cannula served for sampling, and the jugular and portal cannulas for peripheral infusions. The cannulas were tunneled subcutaneously and exteriorized at the back of the neck. They were filled with heparin (1,000 U/ml, Hepalean; Organon Teknika, Toronto, Canada) and were regularly flushed (every 4–5 days) with saline to maintain patency. Circulating heparin activates lipoprotein lipase, which results in release of free fatty acid (FFA) and glycerol. When using heparin as an anticoagulant, we observe an increase in basal FFA vs. free fatty acid (FFA) and glycerol. When using heparin as an anticoagulant, we observe an increase in basal FFA vs.

The SAGlcinf for the study with basal insulin infusion was calculated on Glcinf,Rab, and F estimates of 6.67 pmol/kg plus 0.75 pmol·kg⁻¹·min⁻¹ for PER and POR or half-dose peripheral insulin infusion of 13.5 pmol/kg plus 1.35 pmol·kg⁻¹·min⁻¹ for 1/2 PER. All insulin infusates were prepared in saline containing ~4% (vol/vol) of the dog's own plasma.

During insulin infusion, plasma glucose was clamped at euglycemia with a variable exogenous dextrose infusion (20% dextrose for the high dose and 10% dextrose for the low dose), which was adjusted according to plasma glucose concentration determined every 5 min. The glucose infusion was spiked with [6-3H]glucose tracer according to Finegood et al. (8) to prevent the decline in the glucose specific activity during the glucose clamp and thus minimize errors that are associated with the use of a one-compartment, fixed-pool volume model method for calculations of Ra (8). The amount of tracer in the glucose infusate was based on estimates of suppression of Ra and glucose requirements. The following equation by Finegood et al. (8), modified as in Giaccia et al. (12) to account for partial suppression of Ra, was used to calculate the specific activity of the glucose infusate

\[
S_{A_{Glc_{inf}}} = \frac{I \times [Glc_{inf_{ss}}R_{ab} - F]}{Glc_{inf_{ss}}} \times \frac{1,000}{BW}
\]

where \(S_{A_{Glc_{inf}}}\) is specific activity of the dextrose infusate (dpm/µmol), \(I\) is the constant tracer infusion rate (dpm/min), \(Glc_{inf_{ss}}\) is steady-state glucose infusion rate (µmol·kg⁻¹·min⁻¹), \(R_{ab}\) is basal glucose production (µmol·kg⁻¹·min⁻¹), \(R_{ass}\) is steady-state glucose production (µmol·kg⁻¹·min⁻¹), \(F\) is steady-state suppression of glucose production \([F = (R_{ab} - R_{ass})/R_{ab}]\), and BW is weight of the dog (kg).

The SAGlcinf for the study with basal insulin infusion was calculated on Glcinf,Rab, and F estimates of 6.67 pmol·kg⁻¹·min⁻¹, 12.2 pmol·kg⁻¹·min⁻¹, and 0.4, respectively. The Glcinf,Rab and F values were estimated to be 11.1 pmol·kg⁻¹·min⁻¹ and
0.5 for POR and 1/2 PER groups, respectively, and 22.2 
µmol·kg⁻¹·min⁻¹ and 0.6 for the PER group, respectively, in 
the study with high-rate insulin infusion. The initial esti-
mates, which were based on previous studies (8, 12), were 
updated according to the results in this study. Arterial 
samples were taken every 10 min for 40 min in the basal 
period and then every 10 min in the 1st and 3rd h and every 
15 min in the 2nd h of the hyperinsulinemic clamp. The blood 
samples for [6-³H]glucose (4.0 ml) and insulin analysis (2.0 
ml) were collected in tubes containing sodium fluoride (Fisher, 
Lawn Park, NJ) and dried heparin (50 U/ml of blood). The 
samples for glucagon, FFA, and C-peptide analysis (2.5 ml) 
were collected in tubes containing EDTA (24 mg/dl; Sigma, St. 
Louis, MO) and Trasylol (2,000 kallikrein inhibitory units; 
Bayer, Etobicoke, Canada; 1:1 ratio, 0.1 ml/ml of blood). Blood 
samples for alanine, glycerol, and lactate (1.5 ml) were 
collected in tubes containing an equal volume of 10% percho-
lic acid (BDH, Toronto, Canada). A total blood volume of <150 
ml was withdrawn per experiment. Within 1 h after collec-
tion, the blood samples were centrifuged at 800 g at 4°C. The 
supernatant was stored at −20°C for later analysis.

Laboratory methods. Plasma glucose concentrations were 
measured by the glucose oxidase method on a glucose ana-
lyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, 
CA). The plasma glucose determinations were performed 
during the experiments. The other determinations were 
performed within 3 mo of sample collection. The radioimmu-
noassays for plasma insulin and glucagon have been previ-
ously described (12). Plasma C-peptide levels were deter-
mined by a nonequilibrium double-antibody radioimmunoassay 
procedure (21). The FFA levels were determined with the 
fluorometric method of Miles et al. (18). The plasma concentra-
tions of the gluconeogenic precursors lactate, alanine, and 
glycerol were measured by enzymatic fluorometric methods 
as previously described (12).

For the determination of [6-³H]glucose specific activity, 
plasma was deproteinized in equal volumes of 5% (wt/vol) 
zinc sulfate and 0.3 N barium hydroxide (BDH), which had 
been titrated and adjusted for strength. The supernatant 
was run through columns containing anion and cation ion-
exchange resins (AG 50 W-X8, AG 2-X8; Bio-Rad Laborato-
ries, Richmond, CA) to remove labeled three-carbon metabo-
lites formed from labeled glucose. Ion-exchange resins remove 
charge metabolites such as lactate, pyruvate, and alanine. 
Glycerol is not retained by the resins; however, it has been 
shown that, when using 13C tracers, which, unlike tritiated 
tracers, do not result in labeled water, 99.1% of the radioac-
tivity in the column eluate could be ascribed to glycerol, as it 
was recovered in the potassium gluconate derivative (15). An 
 aliquot of the eluate was evaporated to dryness to eliminate 
tritiated water. After addition of water and liquid scintillation 
solution (ReadySafe; Beckman, Fullerton, CA), the radioac-
tivity from [6-³H]glucose was measured in a beta scintillation 
counter (Canberra Packard, Meriden, CT). Aliquots of the 
infused glucose tracer and of the labeled glucose infusate 
were diluted in nonradioactive plasma of the same dog and 
asayed together with the plasma samples.

Calculations. Rₜ was calculated as an endogenous rate of 
appearance measured with [6-³H]glucose. A modified one-
compartmental model of Steele (8) was used for the calcula-
tions of Rₜ and glucose utilization (Rₚ). This model accounted 
for the exogenously infused mixture of labeled and unlabeled 
glucose. Data were smoothed with the optimal segments 
route using the optimal error algorithm (4). When using 
radio labeled glucose infusates, the monocompartmental as-
sumption becomes minor because the nonsteady part of 
Steele’s equation is close to zero.

The portal insulin levels were calculated using Fick’s 
principle of dye dilution modified by Ader and Bergman (1)

\[ [\text{Ins}]_\text{PO} = \frac{\text{INFI}}{\text{PPF}} + [\text{Ins}]_\text{PE} \]

where [Ins]_PO and [Ins]_PE are portal and systemic insulin 
levels and INFI and PPF are the portal insulin infusion rate 
and portal plasma flow, respectively. The portal plasma flow 
was assumed to be 500 ml/min (23). Initial portal venous 
insulin concentrations were estimated with a value of 2.4 for 
the portal-peripheral insulin gradient (7). The calculation of 
the hepatic sinusoidal insulin levels was based on a 72% 
contribution of the blood flow to the liver from the portal 
circulation and a 28% contribution from the hepatic artery 
(20). We have emphasized the hepatic rather than the portal 
insulin levels because the levels of insulin in the hepatic 
sinusoids (which are vascularized in part by the hepatic 
artery) are the insulin concentrations that interact with the 
hepatocyte insulin receptor. In our calculations, we assumed 
that, in the low-rate insulin infusion studies, endogenous 
insulin secretion was not suppressed during the clamp. This 
was based on the nonsignificant change in plasma C-peptide 
levels. In the high-rate insulin infusion studies, the C-peptide 
levels during the clamp were not significantly different from 
the lower detection limit of the assay in our samples (0.055 
pmol/ml) with all treatments, and the decline from basal was 
>40%. Therefore, we calculated the hepatic insulin levels 
based on a range of suppression of basal endogenous insulin 
secretion from 40 to 100%.

Statistical analysis. The data are expressed as means ± SE. The calculations were performed on the last 90 min of the study. One-way or two-way analysis of variance (ANOVA) for 
repeated measures followed by Tukey’s t-test was carried out 
to determine differences between experimental groups. When 
the experiments were paired (POR, n = 6, vs. 1/2 PER, n = 6, 
at both high- and low-rate insulin infusions), one-way ANOVA 
was used; when experiments were not paired (PER, n = 5, vs. 
POR, n = 6, or 1/2 PER, n = 6, at both high- and low-rate 
insulin infusions), one-way ANOVA was used. For each 
insulin infusion rate, we also repeated the statistical analysis 
of the comparison between VER vs. POR or 1/2 PER after 
excluding the dog that failed to complete the PER experi-
ments. Because the groups were now paired, we could use 
two-way ANOVA on the data from five dogs. The results did 
not change, i.e., 1) at the high-rate insulin infusion, Rₜ 
suppression with PER was greater than that with POR or 1/2 
PER, as expected, and 2) at the low-rate insulin infusion, Rₜ 
suppression with PER was greater than that with 1/2 PER 
but remained nonsignificantly different from that obtained 
with POR. If, for each infusion rate, the comparison between 
POR and 1/2 PER was also performed on only five dogs (those 
that had all 3 experiments), Rₜ suppression with POR re-
maind significantly greater than that with 1/2 PER at the 
Low-rate insulin infusion; however, the difference failed to 
reach significance at the high-rate infusion (P = 0.08). Data 
were also analyzed within each group for differences between 
the experimental periods. Correlations were assessed 
with linear regression analysis. Calculations were performed with 
SAS software (SAS, Cary, NC).

RESULTS

High-rate insulin infusion. The following results 
(means ± SE) are based on a n = 6 for POR and 1/2 PER 
and n = 5 for PER. The peripheral, portal, and hepatic 
insulin levels and the C-peptide levels (absolute values)
Table 1. Plasma insulin and C-peptide levels during PER, POR, or \( \frac{1}{2} \)PER in high-rate insulin infusion study

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>PER</th>
<th>POR</th>
<th>( \frac{1}{2} )PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral insulin, pmol/l</td>
<td>Basal</td>
<td>57.6±4.3</td>
<td>51.6±3.0</td>
<td>50.4±2.4</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>212.4±9.6</td>
<td>119.4±4.8</td>
<td>122.4±4.8</td>
</tr>
<tr>
<td>Estimated portal insulin, pmol/l</td>
<td>40% Suppression</td>
<td>137.6±18.0</td>
<td>123.6±13.2</td>
<td>121.2±10.3</td>
</tr>
<tr>
<td></td>
<td>100% Suppression</td>
<td>263.4±20.4</td>
<td>312.0±16.8</td>
<td>166.6±8.4</td>
</tr>
<tr>
<td>Estimated hepatic insulin, pmol/l</td>
<td>Basal</td>
<td>115.2±15.0</td>
<td>103.2±10.8</td>
<td>101.5±8.6</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>249.6±20.4</td>
<td>256.8±15.3</td>
<td>168.0±8.4</td>
</tr>
<tr>
<td>C-peptide, pmol/ml</td>
<td>Basal</td>
<td>0.11±0.013</td>
<td>0.11±0.019</td>
<td>0.13±0.033</td>
</tr>
<tr>
<td></td>
<td>Clamp</td>
<td>0.049±0.001</td>
<td>0.066±0.012</td>
<td>0.069±0.012</td>
</tr>
</tbody>
</table>

Data are means ± SE. PER, equidose peripheral insulin infusion; POR, portal insulin infusion (2.7 pmol·kg\(^{-1}\)·min\(^{-1}\)); \( \frac{1}{2} \)PER, one-half peripheral insulin infusion.

in the basal state and during the clamp are shown in Table 1. PER induced a rise in peripheral insulin levels that was greater (\( P < 0.001 \)) than that with POR or 1/2 PER, as expected (Fig. 1, top left). As per experimental design, there was no difference in the increase in peripheral insulin levels induced by POR or 1/2 PER. The C-peptide levels allowed us to estimate a 40–100% range of suppression of endogenous insulin secretion, since the decrease from basal was >40% and the clamp values were not significantly different from the lower detection limit of our assay. The increase in the estimated hepatic insulin levels (Fig. 1, top right) was greatest with POR (\( P < 0.05 \) vs. PER), intermediate with PER (\( P < 0.001 \) vs. 1/2 PER), and lowest with 1/2 PER. The results shown in the graph are based on 100% suppression of basal endogenous insulin secretion (which explains why hepatic insulin levels increased minimally with 1/2 PER). However, also assuming only a 40% suppression of basal endogenous insulin secretion, the increase in hepatic insulin levels was greatest with POR, intermediate with PER (\( P < 0.05 \) vs. POR), and least with 1/2 PER (\( P < 0.001 \) vs. POR).

Specific activity of [\( ^{6} \)-\(^{3} \)H\]glucose decreased in all treatments (Table 2); however, the decrease was <25%
of the basal levels. The basal rate of glucose turnover (\(= R_a = R_d \)) was similar between treatments (13.0 ± 0.4, 13.4 ± 0.5, and 12.2 ± 0.6 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) with PER, POR, and 1/2 PER, respectively). Tracer-determined \(R_d\) in the last 90 min of the clamp was 42.7 ± 2.2, 21.5 ± 0.6, and 19.9 ± 1.1 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) in the PER, POR, and 1/2 PER treatments, respectively. As expected, PER increased \(R_a\) more than POR or 1/2 PER (Fig. 2, top). In the clamp period, \(R_a\) was suppressed to 2.7 ± 0.7, 6.3 ± 0.4, and 6.9 ± 0.4 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) (90- to 180-min values) with the PER, POR, and 1/2 PER treatments, respectively. Figure 2, bottom, shows the percent suppression of \(R_a\). PER suppressed \(R_a\) more than POR or 1/2 PER (PER 79 ± 18%, \(P < 0.001\), vs. POR 56 ± 6% or 1/2 PER 45 ± 6%). POR suppressed \(R_a\) slightly more than 1/2 PER (\(P < 0.05\), ANOVA for repeated measures).

With regard to the possible mediators of insulin’s peripheral effect on \(R_a\), the PER, POR, and 1/2 PER treatments suppressed glucagon levels by a similar extent (~15%; Fig. 3, top) from basal levels of 172 ± 25, 150 ± 24, and 158 ± 25 \(\mu\)g/l, respectively; however, FFA levels declined more (\(P < 0.001\)) with PER (57 ± 8%) than POR (33 ± 5%) or 1/2 PER (37 ± 2%) from basal values of 1,165 ± 133, 1,253 ± 82, and 1,164 ± 55 \(\mu\)g/l, respectively (Fig. 3, bottom). Of the gluconeogenic precursors that could also mediate part of the peripheral effect of insulin on \(R_a\), only glycerol decreased to a significantly greater extent with PER (Table 3). Lactate increased with PER and did not change significantly with POR or 1/2 PER (Table 3). Alanine did not change significantly from basal with either treatment (Table 3).

Low-rate insulin infusion. The following results are based on an \(n = 6\) for POR and 1/2 PER and \(n = 5\) for PER. The peripheral, portal, and hepatic insulin levels and the C-peptide levels (absolute values) in the basal state and during the clamp are shown in Table 4. As expected, PER resulted in a greater increase in peripheral insulin levels (\(P < 0.05\)) than POR and 1/2 PER, and the latter two treatments yielded similar increases (Fig. 4, top left). The calculation of the hepatic sinusoidal insulin levels was based on a 0% suppression of endogenous insulin secretion, since the C-peptide levels did not decrease significantly from basal in either treatment. As expected, the increase in the calculated hepatic insulin levels was greatest (\(P < 0.001\)) with POR, intermediate with PER, and lowest (\(P < 0.01\)) with 1/2 PER (Fig. 4, top right). The glucose levels were maintained at constant euglycemia in all treatments (Fig. 4, bottom left). Figure 4, bottom right, shows the glucose infusion rates necessary to maintain euglycemia. PER required the greatest glucose infusion rate, whereas POR and 1/2 PER required similar amounts.

Table 5 shows that plasma glucose specific activity was kept constant. Basal glucose turnover rate (\(= R_g = R_d \)) was similar between treatments (11.5 ± 0.6, 11.9 ± 0.5, and 12.8 ± 0.6 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) with PER, POR, and 1/2 PER, respectively). \(R_d\) increased minimally to 15.5 ± 0.7, 12.9 ± 0.3, and 14.0 ± 0.4 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\), respectively, in the last 90 min of the clamp. As

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### Table 2. Plasma specific activity of [6-\(^3\)H]glucose during PER, POR, or 1/2 PER in high-rate insulin infusion study

<table>
<thead>
<tr>
<th>Time, min</th>
<th>PER</th>
<th>POR</th>
<th>1/2 PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-40</td>
<td>1.60 ± 0.08</td>
<td>1.50 ± 0.10</td>
<td>1.60 ± 0.10</td>
</tr>
<tr>
<td>30-30</td>
<td>1.55 ± 0.05</td>
<td>1.51 ± 0.09</td>
<td>1.54 ± 0.09</td>
</tr>
<tr>
<td>20-22</td>
<td>1.66 ± 0.06</td>
<td>1.55 ± 0.08</td>
<td>1.54 ± 0.08</td>
</tr>
<tr>
<td>10-10</td>
<td>1.60 ± 0.03</td>
<td>1.20 ± 0.12</td>
<td>1.57 ± 0.10</td>
</tr>
<tr>
<td>0-0</td>
<td>1.59 ± 0.04</td>
<td>1.50 ± 0.10</td>
<td>1.50 ± 0.10</td>
</tr>
<tr>
<td>10-10</td>
<td>1.58 ± 0.19</td>
<td>1.50 ± 0.21</td>
<td>1.58 ± 0.21</td>
</tr>
<tr>
<td>20-22</td>
<td>1.48 ± 0.12</td>
<td>1.51 ± 0.16</td>
<td>1.49 ± 0.19</td>
</tr>
<tr>
<td>30-1</td>
<td>1.30 ± 0.14</td>
<td>1.40 ± 0.18</td>
<td>1.41 ± 0.20</td>
</tr>
<tr>
<td>50-50</td>
<td>1.39 ± 0.12</td>
<td>1.40 ± 0.13</td>
<td>1.40 ± 0.14</td>
</tr>
<tr>
<td>75-75</td>
<td>1.30 ± 0.06</td>
<td>1.36 ± 0.16</td>
<td>1.42 ± 0.10</td>
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<tr>
<td>90-90</td>
<td>1.20 ± 0.17</td>
<td>1.34 ± 0.19</td>
<td>1.31 ± 0.10</td>
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<tr>
<td>105-105</td>
<td>1.23 ± 0.13</td>
<td>1.25 ± 0.83</td>
<td>1.33 ± 0.11</td>
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<td>120-120</td>
<td>1.17 ± 0.12</td>
<td>1.31 ± 0.19</td>
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<td>130-130</td>
<td>1.16 ± 0.15</td>
<td>1.26 ± 0.16</td>
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<td>140-140</td>
<td>1.16 ± 0.16</td>
<td>1.26 ± 0.12</td>
<td>1.29 ± 0.15</td>
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<td>150-150</td>
<td>1.19 ± 0.11</td>
<td>1.20 ± 0.88</td>
<td>1.22 ± 0.52</td>
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<td>160-160</td>
<td>1.18 ± 0.13</td>
<td>1.24 ± 0.13</td>
<td>1.28 ± 0.55</td>
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<td>170-170</td>
<td>1.16 ± 0.14</td>
<td>1.24 ± 0.15</td>
<td>1.25 ± 0.47</td>
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<tr>
<td>180-180</td>
<td>1.21 ± 0.14</td>
<td>1.26 ± 0.12</td>
<td>1.35 ± 0.36</td>
</tr>
</tbody>
</table>

Data are means ± SE in disintegrations·min\(^{-1}\)·dpm·µmol\(^{-1}\).

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Fig. 2. Percent change in glucose utilization from basal period (top) and percent change in glucose production (bottom) in the 3 experimental groups in high-rate insulin infusion study. Experimental design is outlined in Fig. 1. Values are presented as means ± SE. Basal levels are reported in RESULTS.
expected, the PER treatment increased Ra more than POR or 1/2 PER (Fig. 5, top).

The Ra values in the clamp period (last 90 min) were suppressed to 6.1 ± 0.4, 6.9 ± 0.3, and 7.8 ± 0.3 µmol·kg\(^{-1}\)·min\(^{-1}\) with the PER, POR, and 1/2 PER treatments, respectively. The percent suppression of Ra is shown in Fig 5, bottom. Ra suppression was similar with PER (46 ± 9%) and POR (43 ± 4%) and significantly less with 1/2 PER (36 ± 8%, P < 0.01 vs. POR, ANOVA for repeated measures).

Glucagon levels declined by a similar extent (~15%; Fig. 6, top) from basal levels of 173 ± 29, 134 ± 16, and 172 ± 21 pmol/l in PER, POR, and 1/2 PER groups, respectively. The FFA levels declined by 29 ± 10, 26 ± 4, and 21 ± 6% from a basal level of 1,145 ± 72, 1,260 ± 43, and 1,013 ± 49 µmol/l in the PER, POR, and 1/2 PER groups, respectively (Fig. 6, bottom). Unlike the high-rate insulin infusion, there were no significant differences between treatments. Alanine and lactate did not change from basal levels, and glycerol levels decreased minimally but not significantly (Table 6).

**Table 4. Plasma insulin and C-peptide levels during PER, POR, or 1/2 PER in low-rate insulin infusion study**

<table>
<thead>
<tr>
<th>Perio</th>
<th>PER</th>
<th>POR</th>
<th>1/2 PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>56.3 ± 3.6</td>
<td>46.0 ± 2.3</td>
<td>48.6 ± 5.7</td>
</tr>
<tr>
<td>Clamp</td>
<td>94.2 ± 7.5</td>
<td>68.6 ± 3.6</td>
<td>66.7 ± 5.3</td>
</tr>
<tr>
<td>Basal</td>
<td>135.2 ± 14.2</td>
<td>110.4 ± 10.0</td>
<td>116.5 ± 23.6</td>
</tr>
<tr>
<td>Clamp</td>
<td>170.6 ± 16.4</td>
<td>171.6 ± 12.5</td>
<td>134.4 ± 20.1</td>
</tr>
<tr>
<td>Basal</td>
<td>113.2 ± 11.9</td>
<td>92.4 ± 8.4</td>
<td>97.4 ± 19.7</td>
</tr>
<tr>
<td>Clamp</td>
<td>148.5 ± 14.1</td>
<td>142.0 ± 10.9</td>
<td>115.6 ± 16.8</td>
</tr>
<tr>
<td>Basal</td>
<td>0.13 ± 0.021</td>
<td>0.12 ± 0.022</td>
<td>0.090 ± 0.010</td>
</tr>
<tr>
<td>Clamp</td>
<td>0.12 ± 0.032</td>
<td>0.11 ± 0.029</td>
<td>0.080 ± 0.013</td>
</tr>
</tbody>
</table>

Data are means ± SE.

**DISCUSSION**

In normal dogs at high physiological insulin concentrations, PER suppressed tracer-determined Ra more than equidose POR or 1/2 PER. Because peripheral insulin levels were greater in PER, whereas hepatic insulin levels were lower in PER vs. POR, this result is consistent with a peripheral effect of insulin in suppressing Ra. However, in the normal dogs at both low and high physiological insulin concentrations, POR suppressed Ra more than 1/2 PER despite peripheral insulin levels being matched in both studies. Because hepatic insulin levels were greater with POR than with 1/2 PER, this result is consistent with a direct hepatic effect of insulin in suppressing Ra. The novelty of the present study is the combination of the following two findings, both obtained for the first time during euglycemic clamps without using somatostatin: 1) in normal dogs, both hepatic and peripheral effects of insulin contribute to Ra suppression, and 2) the contribution of the peripheral effect predominates (we have addressed the second point in the end of the DISCUSSION).

In the low-rate insulin infusion study, plasma glucose specific activity remained constant. In the high-rate insulin infusion study, specific activity declined by ~25% with PER, more than with POR or 1/2 PER; therefore, we cannot exclude that some underestimation of Ra occurred. However, we (9) and others (14) have found that, provided the decline in specific activity is slow and <30% of the basal levels, the underestimation is negligible. In addition, we have repeated our calculation on the steady-state specific activities of the last hour of the clamp using the steady-state formula. The resulting values for Ra suppression were similar to those reported here (high rate: PER 79 ± 15%, POR
At both low and high insulin concentrations, Rₐ was proportional to the peripheral insulin levels, as expected. Similar to Rₐ, the glucose infusion rate required to maintain euglycemia was greatest with PER but similar with POR and 1/2 PER despite Ra suppression being greater with POR than with 1/2 PER. This discrepancy is likely to be explained by the fact that small changes in Ra represent a sizable percentage of basal Ra, whereas they represent only a small fraction of the glucose requirements during a clamp.

At both insulin infusion rates, POR suppressed Rₐ more than 1/2 PER (high rate, \( P < 0.01 \), low rate, \( P < 0.05 \), two-way ANOVA for repeated measures). Because the peripheral insulin levels were similar between POR and 1/2 PER, the difference in suppression should be ascribed to the difference in the hepatic insulin levels and is therefore consistent with a direct hepatic effect of insulin on Rₐ. With high-rate insulin infusion, Rₐ suppression was greater with PER than with POR. With low-rate insulin infusion, Rₐ suppression was not significantly different between PER and POR; however, because estimated hepatic insulin levels were greater with POR than with PER, one would expect a greater Rₐ suppression with POR. Therefore, even a similar degree of suppression by the two treatments implies that insulin suppressed Rₐ by a peripheral effect. Because Rₐ suppression was greater with PER than with POR at the high but not the low insulin infusion concentration,
rate, our data suggest that the relative contribution of the peripheral effect is greater at higher insulin concentrations.

Insulin-induced inhibition of FFA levels has been found to play a dominant role in the peripheral effect of insulin on Ra (16, 24, 28). The present study is consistent with these results, as FFA levels decreased more with PER than with POR or 1/2 PER at the high-rate insulin infusion where Ra suppression was greater with PER. If the percent suppression of Ra was correlated with that of FFA as previously reported (23), the steady-state values were significantly correlated at the high-rate insulin infusion ($r = 0.69, P < 0.01, n = 17$ experiments) and almost significantly correlated at the low-rate infusion ($r = 0.48, P = 0.051, n = 17$).

The time course of Ra suppression was significantly correlated with that of FFA at both the high-rate insulin infusion ($r = 0.73, P < 0.001, n = 21$) and the low-rate infusion ($r = 0.82, P < 0.001, n = 21$). It might be argued that, at the high-rate infusion, the insulin-induced decrease in FFA levels did not appear to precede Ra suppression. This suggests, in our view, that insulin's suppression of Ra is partly independent of FFA (early Ra suppression may be mediated by insulin's hepatic effect (27)). However, the difference in FFA between high-dose PER and POR did precede the difference in Ra, suggesting that FFA suppression is linked to the peripheral effect of insulin on Ra. In keeping with this observation, decreased FFA levels obtained with antilipolytic agents, such as acipimox, have been shown to enhance Ra suppression during hyperinsulinemic clamps (10, 25).

The actions of peripheral insulin in suppressing Ra may include decreased availability of gluconeogenic precursors, i.e., alanine and glycerol, due to insulin's suppression of proteolysis and lipolysis. In depancreatized dogs (12), we observed slight differences in ala-

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<table>
<thead>
<tr>
<th>Period</th>
<th>PER</th>
<th>POR</th>
<th>1/2PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Basal</td>
<td>328.7 ± 33.0</td>
<td>411.0 ± 35.9</td>
<td>406.4 ± 21.9</td>
</tr>
<tr>
<td>Clamp</td>
<td>335.9 ± 14.4</td>
<td>343.4 ± 20.3</td>
<td>356.9 ± 18.5</td>
</tr>
<tr>
<td>Alanine Basal</td>
<td>247.4 ± 21.5</td>
<td>285.0 ± 21.4</td>
<td>306.8 ± 22.1</td>
</tr>
<tr>
<td>Clamp</td>
<td>263.5 ± 11.8</td>
<td>302.4 ± 13.1</td>
<td>303.1 ± 18.9</td>
</tr>
<tr>
<td>Glycerol Basal</td>
<td>91.2 ± 3.5</td>
<td>107.2 ± 5.6</td>
<td>91.4 ± 7.3</td>
</tr>
<tr>
<td>Clamp</td>
<td>82.1 ± 4.1</td>
<td>89.2 ± 7.3</td>
<td>82.4 ± 5.7</td>
</tr>
</tbody>
</table>

Data are means ± SE in µmol/l.
nine and glycerol levels between treatments; however, we calculated that these differences could have only minimally contributed to the effect of peripheral insulin on $R_a$. In the present study, only glycerol was significantly different between treatments at the high insulin infusion rate.

In depancreatized dogs, we have shown that glucagon may be an important mediator of the peripheral effect of exogenous insulin on $R_a$ (11). In the present study, steady-state $R_a$ suppression was correlated with steady-state glucagon suppression at both insulin infusion rates (high rate: $r = 0.57$, $P < 0.05$, $n = 17$; low rate: $r = 0.63$, $P < 0.01$, $n = 17$) and the time course of $R_a$ suppression was correlated with that of glucagon suppression at the low insulin infusion rate ($r = 0.75$, $P < 0.001$, $n = 21$). However, some degree of correlations between variables that are both affected by insulin is to be expected. Figures 3 and 6 show that there was no difference in glucagon levels between any of the three treatments at either insulin infusion rate, indicating that suppression of glucagon may not play a major role in the peripheral effect of insulin in normal dogs.

The difference between normal and diabetic dogs might relate to the presence of residual B cell insulin secretion directly inhibiting A cell glucagon secretion in normal dogs, whereas, in the absence of endogenous insulin secretion in depancreatized dogs, insulin-induced inhibition of glucagon is only proportional to the insulin levels in normal dogs.

The results of this protocol are in accordance with our study in humans, where with the use of a high-rate tolbutamide-induced insulin secretion (0.88 mU·kg$^{-1}$·min$^{-1}$), both hepatic and peripheral effects of insulin in suppressing $R_a$ were found (17). At the high-rate insulin infusion in dogs, the percent difference in suppression of $R_a$ per unit difference in the rise in hepatic insulin levels (POR vs. 1/2 PER) was 11/104 = 0.11%/pM. Taking into account that the rise in hepatic insulin levels was 24 pM greater in POR than in PER, the percent difference in suppression of $R_a$ per unit difference in the rise in peripheral insulin levels (PER vs. POR) was [(0.11 × 24)/23/90 = 0.28%/pM. At the low-rate insulin infusion in dogs, the percent difference in suppression of $R_a$ per unit difference in the rise in hepatic insulin levels (POR vs. 1/2 PER) was 7/32 = 0.22%/pM. Taking into account that the rise in hepatic insulin levels was 15 pM greater in POR than in PER, the percent difference in suppression of $R_a$ per unit difference in the rise in peripheral insulin levels (PER vs. POR) was [(0.22 × 15) + 3]/15 = 0.42%/pM. Thus the potency of peripheral insulin per unit concentration was 0.28/0.11 = 2.6 greater than that of hepatic insulin at the high-rate insulin infusion and 0.42/0.22 = 1.9 greater than that of hepatic insulin at the low-rate insulin infusion. These data suggest that the peripheral effect of insulin prevail over the hepatic effect at both low and high insulin concentrations and its relative contribution to suppression of $R_a$ may increase with the insulin dose. If the same calculations are performed with our data in humans (17), the results show that portal insulin levels were approximately threefold more potent than hepatic levels in suppressing $R_a$.

These results are affected by the assumption used in calculating the hepatic insulin levels. If our calculated portal insulin levels are compared with those determined directly by Sindelar et al. (27), it would appear that we might have underestimated the differences in portal insulin levels (and therefore hepatic insulin levels) between POR and PER or 1/2 PER. If the difference in hepatic insulin levels was underestimated, we could have overestimated the effect of hepatic insulin. This would strengthen our conclusion about the predominance of the peripheral effect of insulin vs. the hepatic effect.

In contrast, Sindelar et al. have reported that a selective rise of 96 pmol in either portal or peripheral insulin levels resulted in similar suppression of $R_a$, consistent with equal potency of insulin delivered by either route per unit insulin concentration. The discrepancy between our results and theirs may relate to the use of the pancreatic clamp technique (somatostatin infusion + glucagon replacement) in their study. As previously discussed, we cannot exclude that somatostatin might alter the balance between insulin's hepatic and peripheral effects. In addition, we (11) and other authors (19) have found that glucagon (which was allowed to decrease in our study but is kept constant during pancreatic clamps) potentiates the direct effect
of insulin. However, it is unlikely that the pancreatic clamp technique is the main reason for the quantitative discrepancy between Sindelar’s study and ours, since, in Ader and Bergman’s (1) study, pancreatic clamps were used, but Ra suppression was even more dependent on peripheral insulin than in our study. Alternatively, the discrepancy between Sindelar’s data and ours may be due to the fact that, in our experiments, similar to those of Ader and Bergman’s, hepatic and peripheral insulin levels rise simultaneously, whereas, in Sindelar’s protocol, a selective rise in either portal or peripheral insulin levels was obtained. The latter authors have recently shown that the effects of portal and peripheral insulin are synergistic on hepatic glucose uptake and are additive on net hepatic glucose balance but not on tracer-determined Ra (29). Thus it is possible that, when both levels rise, as occurs physiologically, the effect of peripheral insulin on Ra predominates, thus masking insulin’s hepatic effect.

We have previously reported that, in depancreatized diabetic dogs, we could only detect a peripheral effect of insulin on Ra (12). Thus it appears that insulin’s hepatic effect may be abolished or masked by the peripheral effect under diabetic conditions.

In conclusion, at both low and high physiological insulin levels in normal dogs, insulin can suppress Ra by both peripheral and hepatic effects. Quantitatively, the peripheral effect predominates, being at least two times as potent as the hepatic effect.

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