Increased Dependence of Glucose Production on Peripheral Insulin in Diabetic Depancreatized Dogs

Adria Giacca, Richard McCall, Bernie Chan, and Zhi Qing Shi

We have recently found that in nondiabetic dogs and humans, suppression of glucose production (GP) is mediated by both peripheral and hepatic effects of insulin. We have also found that both nonesterified fatty acids (NEFA) and glucagon are important determinants of the peripheral effect of insulin on GP. However, in moderately hyperglycemic depancreatized dogs, suppression of GP appeared to be mediated by peripheral but not hepatic insulin. In this latter study, insulin concentrations were in the high postprandial range (~300 pmol/L) and suppression of GP may have been close to maximum. The aim of the present study was to determine whether GP can be regulated by hepatic insulin in depancreatized dogs at low insulin concentrations in the postabsorptive range. Depancreatized dogs were maintained at moderately hyperglycemic levels (~10 mmol/L) by subbasal insulin infusions. In paired experiments, additional low-dose equimolar insulin infusions (0.75 pmol/kg · min) were administered peripherally (PER, n = 6) or portally (POR, n = 6) during glucose clamps. This resulted in a minimal increase in peripheral insulin levels, which was greater in PER versus POR, 29.0 ± 3.7 versus 11.7 ± 2.2 pmol/L. Also, we infused insulin peripherally at half this rate (½PER, n = 6) to match the increase in peripheral insulin levels in POR (½PER, 14.6 ± 2.2) and thus obtain a selective POR versus ½ PER difference in hepatic sinusoidal insulin levels. PER suppressed GP more than POR (45.4% ± 4.0% *v* 35.3% ± 6.8%, $P < .001$), whereas POR did not suppress GP more than ½ PER (35.6% ± 6.3%). Therefore, suppression of GP was proportional to peripheral rather than hepatic sinusoidal insulin levels, as in our previous study at higher insulin concentrations. In conclusion, during glucose clamps in moderately hyperglycemic depancreatized dogs, (1) suppression of GP was dominated by insulin's peripheral effects not only at postprandial but also postabsorptive insulin levels, and (2) we found no evidence for a hepatic effect of insulin in suppressing GP. We hypothesize that this effect is reduced in the depancreatized dog model of diabetes due to hepatic insulin resistance and/or hyperglycemia.

IN NORMAL PHYSIOLOGY, insulin is secreted into the portal circulation, and is degraded by about 50% on its first pass through the liver. The hormone that escapes hepatic degradation is then diluted in the systemic circulation. Thus, the hepatic degradation and peripheral dilution of insulin maintain a portal-peripheral insulin concentration gradient. Since the liver is vascularized 70% to 80% by the portal vein and 20% to 30% by the hepatic artery,[1] hepatic sinusoidal levels of insulin are greater than the peripheral levels. It is known that insulin has a strong inhibitory effect on glucose production (GP), but the extent to which this represents a direct action of hepatic sinusoidal insulin via insulin's interactions with its hepatocyte receptor or an action of peripheral insulin via its effects on extrahepatic tissues is still unclear.[2-8] Recent studies have emphasized the importance of the peripheral action of insulin in inhibiting GP in normal dogs[9-11] and its linkage to the insulin-induced suppression of lipolysis,[11,12] whereas other studies have emphasized the importance of insulin's direct hepatic action.[13,14] In our studies in normal dogs and humans, the regulation of GP was both peripheral and hepatic.[15,16]

In insulin-treated diabetes, insulin is injected subcutaneously and absorbed into the peripheral circulation. Thus, hepatic sinusoidal insulin levels are equal to the peripheral insulin levels. In the absence of a portal-peripheral insulin concentration gradient, achievement of normoglycemia may not be possible without peripheral hyperinsulinemia. This is thought to increase hepatic insulinization, thereby inhibiting GP by a direct action. According to this concept, the peripheral replacement of insulin is a major cause of peripheral hyperinsulinemia. Hyperinsulinemia has been shown to be an independent risk factor for cardiovascular disease,[17,18] although the relative role of hepatic versus peripheral hyperinsulinemia in atherogenesis is not entirely clear. However, the peripheral replacement of insulin would not contribute to peripheral hyperinsulinemia if suppression of GP in diabetes is mediated by a peripheral rather than hepatic effect of insulin.

We have addressed this question in a previous study[19] in moderately hyperglycemic depancreatized dogs. We found that suppression of GP was proportional to peripheral rather than hepatic insulin levels, in contrast to our results in nondiabetic dogs, where GP suppression was mediated by both peripheral and hepatic insulin.[15] However, nondiabetic dogs were studied at lower insulin infusion rates (2.7 and 0.75 pmol/kg · min) than depancreatized dogs (5.4 pmol/kg · min). Since suppression of GP is very sensitive to insulin, at the insulin levels achieved in depancreatized dogs (high postprandial range), GP may still remain sensitive to peripheral insulin but be almost completely suppressed by hepatic insulin. In alloxan-diabetic dogs, low insulinization accentuated a direct hepatic effect of insulin in suppressing GP under conditions of declining glucose.[2] Also in our glucose clamp studies in normal dogs,[15] the direct effect of insulin appeared to be more accentuated at 0.75 versus 2.7 pmol/kg · min insulin infusion.

In the present study, we used low insulin infusion rates equal

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to those administered to normal dogs (0.75 pmol/kg · min) to determine whether we could detect a direct hepatic effect of insulin on GP in moderately hyperglycemic depancreatized dogs at levels of insulin close to the fasting range. This would imply that in insulin-treated diabetes, the nonphysiological route of insulin delivery, which may not be associated with peripheral hyperinsulinemia in the postprandial state, would still be a cause of peripheral hyperinsulinemia in the fasting state.

MATERIALS AND METHODS

Experimental Animals and Preparation

The study was performed on six postabsorptive depancreatized male dogs (model of type 1 diabetes mellitus) weighing 22 to 34 kg. Depancreatized dogs are a model of selective insulin deficiency, because glucagon (IRG 3500) is secreted by oxyntic cells in the gastric mucosa of dogs.[20] Pancreatic and extrapancreatic glucagon has been found to have identical biological effects on glycogenolysis and gluconeogenesis.[21] Total pancreatectomy was performed under general anesthesia induced with sodium thiopental (Abbott Laboratories, Montreal, Quebec, Canada) and maintained with 0.5% halothane (Halocarbon Laboratories, River Edge, NJ) with nitrous oxide (Canox, Toronto, Ontario, Canada) and assisted ventilation. The pancreas was completely removed with care taken to preserve duodenal vascularization through the pancreatoduodenal vessels.

A silastic cannula (0.04 in ID; Baxter Healthcare, McGaw Park, IL) was inserted into the portal vein through a branch of the splenic vein and advanced until the tip was approximately 1.0 cm beyond the confluence of the splenic vein with the portal vein, ie, approximately 5 cm from the branching point of the portal vein into its left and right bifurcations to the liver. In the present study, we did not have sampling catheters in the portal and hepatic vein, because the failure rate of these catheters is high in dogs undergoing repeated experiments and the catheterization procedure may be too invasive if combined with a pancreatectomy. Three silastic cannulae (one 0.04 in ID and two 0.03 in ID) were inserted into a jugular vein and advanced into the superior vena cava. In addition, a silastic cannula (0.04 in ID) was inserted into a carotid artery and advanced into the aortic arch. The carotid cannula served for arterial sampling, and the jugular and portal cannulae served for infusions. The cannulae were tunneled subcutaneously and exteriorized at the back of the neck. They were filled with heparin (1,000 U/mL Hepalean; Organon Teknika, Toronto, Ontario, Canada) and bandaged around the dog's neck. The cannulae were regularly flushed (every 4 to 5 days) with saline to maintain patency. Dogs received a diet of 400 g dry chow (Purina Mills, St Louis, MO) and 670 g canned meat (Derby Pet Food, Brampton, Ontario, Canada) once per day. Pancreatic enzyme capsules (Cotazym; Organon Canada, Toronto, Ontario, Canada), iron, and folic acid tablets were mixed with the food. Regular and NPH porcine insulin (Eli Lilly & Co, Indianapolis, IN) was injected subcutaneously at meal time to maintain glycosuria less than 1%. Porcine insulin does not induce the formation of anti-insulin antibodies for at least 2 months,[22] thus allowing accurate measurement of plasma insulin levels.

Only dogs who did not lose weight, had normal body temperature, were consuming regularly their daily food ration, had no diarrhea or visible steatorrhea, and had at least 3 days of relatively well-controlled diabetes (urine glucose < 1%) were allowed to undergo experiments. The hematocrit level of the dogs at the time of the experiment was 40% ± 1%. The dogs received the normal amount of food the day before the experiment. The regular insulin dose was unaffected, and the NPH insulin was reduced to half or one third of the previous day's dose. The experiments were performed after an overnight fast of at least 18 hours. The animal model and preparation were the same as in our previous study.[19] All procedures were in accordance with the Canadian Council of Animal Care Standards and were approved by the Animal Care Committee of the University of Toronto.

Experimental Design

Three paired experiments were performed in each dog in random order: (1) portal insulin infusion (POR), (2) equidose peripheral insulin infusion (PER), and (3) half-dose peripheral insulin infusion (½ PER). As in our previous studies in depancreatized[19] and normal[15] dogs, POR and ½ PER treatments were designed to result in matched peripheral insulin levels and therefore generate a selective difference in hepatic sinusoidal insulin levels. This difference should induce a difference in GP in the presence of a significant direct effect of insulin. The PER treatment results in higher peripheral and lower hepatic sinusoidal insulin levels than the POR treatment. The comparison between POR and PER is therefore mainly indicative of a peripheral effect of insulin. We performed this treatment mainly to confirm that the peripheral effect of insulin on GP was still detectable despite the small increase in insulin levels obtained with our low-rate insulin infusions.

On the morning of the experiment, the dogs were markedly hyperglycemic due to the reduction of the insulin dose on the previous day (PER, 23.7 ± 1.2 mmol/L; POR, 25.5 ± 2.9; ½ PER, 22.8 ± 1.3; Fig 1). At the onset of each experiment, regular porcine insulin was infused intraportally initially at a high dose (Fig 1) to decrease plasma glucose over time. It required about 4 to 5 hours of variable insulin infusion (PER, 280 ± 42 minutes; POR, 310 ± 53; ½ PER, 273 ± 57) to obtain

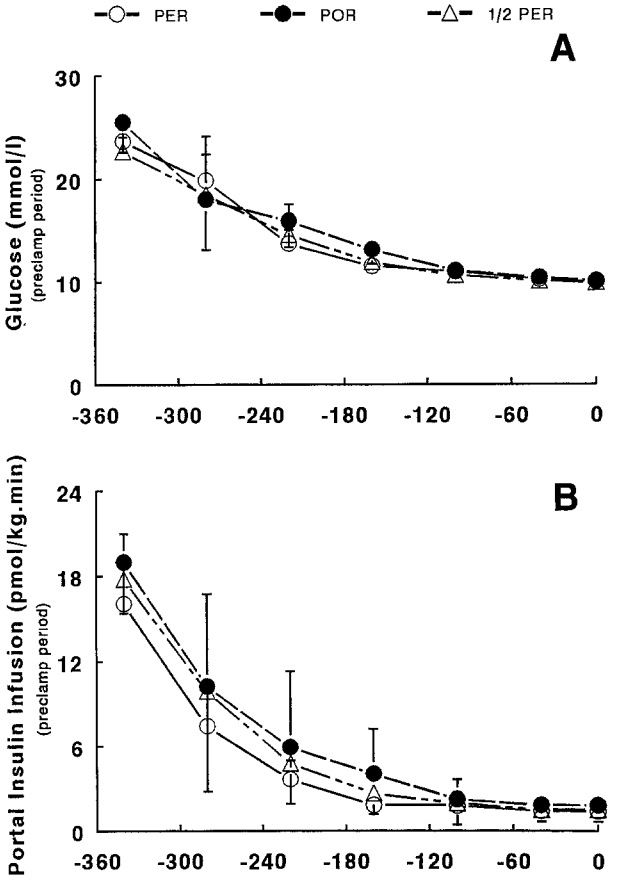

Fig 1. Plasma glucose levels (A) and portal insulin infusion rates (B) in the preclamp period in the 3 experimental groups. The same insulin infusion rates as given in the −70 to 0 minute period were continued during the glucose clamp. Experimental design is outlined in the legend to Fig 2. Values are presented as the mean ± SEM.

constant moderate hyperglycemia (9 to 11 mmol/L). During the first 1 to 2 hours, the insulin infusion rates were high; thereafter, they were close to basal replacement rates. There was no significant difference in the glucose level or insulin infusion rate between the treatments at any point. When glucose levels declined to less than 14 mmol/L, a bolus of 6-[^{3}H]glucose (7.77×10^7 dpm; New England Nuclear, Boston, MA) was administered and a continuous 6-[^{3}H]glucose infusion (5.55×10^5 dpm/min) was initiated (time −160 minutes) to enable the measurement of glucose turnover. The tracer equilibration period was at least 120 minutes. We used 6-[^{3}H]glucose because this tracer was used in our previous studies in depancreatized[19] and normal[15] dogs. After at least 30 minutes of steady-state glucose levels with a fixed low-dose portal insulin infusion (1.33 ± 0.16, 1.70 ± 0.27, and 1.50 ± 0.39 pmol/kg · min in PER, POR, and ½ PER treatments, respectively, NS; Fig 1), basal samples were taken every 10 minutes (from −40 to 0 minutes). At time 0 minutes, an additional low-dose infusion of insulin was administered either portally (POR, 7.5 pmol/kg + 0.75 pmol/kg · min) or peripherally (PER, 7.5 pmol/kg + 0.75 pmol/kg · min) at the same rate or peripherally at half the rate (½ PER, 3.75 pmol/kg + 0.375 pmol/kg · min). The ½ PER treatment was designed to match peripheral insulin levels obtained with the POR treatment and thus obtain a selective difference in hepatic insulin levels. Except for the lower insulin infusion rates, the experimental design and procedures were identical to those of our previous study in depancreatized dogs.[19] As in the previous study in depancreatized dogs,[19] as well as our study in normal dogs,[15] small boluses of insulin corresponding to the infusion rate given in 10 minutes were administered at time 0 to rapidly achieve a plateau in insulin levels.[23] All insulin infusions were prepared in saline containing approximately 4% (vol/vol) of the dog's own plasma. Plasma glucose was clamped at the initial preclamp glycemic levels for 3 hours with a variable 10% dextrose infusion adjusted according to plasma glucose concentrations determined every 5 minutes. The glucose infusion was spiked with 6-[^{3}H]glucose according to the method of Finegood et al[24] to prevent the decline in glucose specific activity during the glucose clamp and thus minimize errors associated with the use of a one-compartment fixed-pool volume model method for calculations of GP.[24,25] The 6-[^{3}H]glucose was HPLC-purified to remove contaminants, which have also been shown to induce errors in the determination of GP.[26] The following equation by Finegood et al[24] as modified by Giacca et al[19] to account for partial suppression of GP was used to calculate specific activity of the dextrose infusate:

$$\text{SAginf} = \frac{I \times \{[\text{GINF(ss)}/\text{Ra(b)}] - F\}}{\text{GINF(ss)}} \times \frac{1{,}000}{\text{BW}}.$$

SAginf is the specific activity of the glucose infusate (dpm/μmol), I is the constant tracer infusion rate (dpm/min), GINF(ss) is the steady-state glucose infusion rate (μmol/kg · min), Ra(b) is the basal GP (μmol/kg · min), F is the steady-state suppression of GP, F = [Ra(b) − Ra(ss)]/Ra(b), Ra(ss) is the steady-state GP (μmol/kg · min), and BW is the weight of the dog (kg).

The SAginf was based on GINF, Ra(b), F initial estimates of 6.67 μmol/kg · min, 15.0 μmol/kg · min, and 40%, respectively. These estimates were based on our previous[19] and pilot studies, and were continuously updated according to the experimental results.

Arterial samples were taken every 10 minutes in the first and third hour and every 15 minutes in the second hour of the hyperinsulinemic clamp. Blood samples for 6-[^{3}H]glucose and insulin analysis were collected in tubes containing sodium fluoride (Fisher, Fair Lawn, NJ) and dried heparin. Samples for glucagon and NEFA analysis were collected in tubes containing EDTA (Sigma, St Louis, MO) and Trasylol (10,000 Kallikrein IU/mL; Bayer Inc, Etobicoke, Ontario, Canada). Blood samples for alanine, glycerol, and lactate were collected in tubes containing an equal volume of 10% perchloric acid (BDH, Toronto, Ontario, Canada). Within 1 hour after collection, the 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[27] on a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma insulin levels were determined by radioimmunoassay[28] using an antibody provided by Dr P.H. Wright (Indianapolis, IN). The glucagon radioimmunoassay was performed according to the method of Faloona and Unger.[29] The O4A antiserum was obtained from Dr Unger (Dallas, TX) and is specific for the glucagon C-terminal region. NEFA concentrations were determined with a radiochemical technique.[30] Lactate, alanine, and glycerol levels were determined by enzymatic fluorometric methods.[31]

For the determination of 6-[^{3}H]glucose specific activity, plasma was deproteinized in equal volumes of 5% (wt/vol) zinc sulfate and 0.3N barium hydroxide (BDH) that was titrated. The supernatant was passed through columns containing anion and cation ion-exchange resins (AG 50 W-X8 and AG 2-X8; Biorad Laboratories, Richmond, CA) to remove labeled 3-carbon metabolites formed from labeled glucose. A 1-mL aliquot of the eluate was then evaporated to dryness to eliminate tritiated water. After addition of water and liquid scintillation solution (ReadySafe, Beckman), radioactivity from 6-[^{3}H]glucose was measured in a beta-scintillation counter (Camberra Packard, Meriden, CT). Aliquots of the infused glucose tracer and of the labeled glucose infusate were diluted with nonradioactive plasma of the same dog and assayed together with the plasma samples.

Calculations

GP was calculated as the endogenous rate of appearance measured with 6-[^{3}H]glucose. Glucose utilization represents metabolic glucose utilization, since in the present study plasma glucose levels were less than the renal glucose threshold of dogs, which is approximately 13 mmol/L.[32] Glucose and glucose specific activity data were smoothed with the optimal segments routine using the optimal error algorithm.[33,34] For the calculation of GP and glucose utilization, a modified[24] one-compartmental Steele model[35] was used to account for the exogenously infused mixture of labeled and unlabeled glucose. When using radiolabeled glucose infusate, the monocompartmental assumption becomes minor because the non–steady-state part of Steele's equation is close to zero.

We calculated portal insulin levels using the Fick principle of dye dilution modified by Ader and Bergman[9]:

$$[\text{Ins}]_{\text{PO}} = \frac{\text{INF}_{\text{POR}}}{\text{PPF}} + [\text{Ins}]_{\text{PE}}.$$

$[\text{Ins}]_{\text{PO}}$ and $[\text{Ins}]_{\text{PE}}$ are portal and peripheral insulin levels, respectively. INF_{POR} and PPF are the portal insulin infusion rate and portal plasma flow. The portal plasma flow was assumed to be 500 mL/min.[10] Initial portal insulin concentrations were estimated with a value of 2.4 for the portal-peripheral insulin gradient.[36] The calculation of hepatic sinusoidal insulin levels was based on a 72% contribution of the blood flow to the liver by the portal circulation and a 28% contribution by the hepatic artery.[1] We have emphasized the hepatic sinusoidal rather than portal insulin levels because insulin levels in the hepatic sinusoids (which are vascularized in part by the hepatic artery)

are the insulin concentrations that interact with the hepatocyte insulin receptor. Portal and hepatic sinusoidal insulin levels were also calculated using the method of De Feo et al[36]:

$$[\text{Ins}]_{\text{PO}} = [\text{Ins}]_{\text{PE}} + ([\text{Ins}]_{\text{PO0}} - [\text{Ins}]_{\text{PE0}}) \times \frac{\text{INF}_{\text{POR}}}{\text{INF}_{\text{POR0}}}.$$

The 0 subscript refers to time 0 minutes. The results were very similar to those obtained using the Fick principle (Table 1).

We calculated the first-pass hepatic insulin extraction (HIE) of portally delivered insulin using the following formula:

$$\text{HIE}\ (\%) = \frac{\Delta\text{INF}_{\text{POR}} - (\Delta[\text{Ins}]_{\text{PE-POR}} \times \text{INF}_{1/2\text{PER}}/\Delta[\text{Ins}]_{\text{PE-1/2PER}})}{\Delta\text{INF}_{\text{POR}}} \times 100.$$

$\Delta\text{INF}_{\text{POR}}$ is the additional portal insulin infusion during the POR treatment (7.5 pmol/kg · min), $\Delta[\text{Ins}]_{\text{PE-POR}}$ is the increment in the peripheral insulin level due to $\Delta\text{INF}_{\text{POR}}$, $\text{INF}_{1/2\text{PER}}$ is the ½ PER insulin infusion in the paired ½ PER treatment in the same dog, and $\Delta[\text{Ins}]_{\text{PE-1/2PER}}$ is the increment in peripheral insulin due to $\text{INF}_{1/2\text{PER}}$.

Statistics

The data are expressed as the mean ± SEM. Two-way ANOVA for repeated measures followed by Tukey's *t* test was performed to determine differences among experimental groups in the basal (−40 to 0 minutes) and 0- to 180-minutes periods of the clamp. Data were also analyzed within each group to determine differences between the experimental periods. Calculations were performed with SAS software (SAS Statistical Analysis System, Cary. NC).

RESULTS

The following results are based on six paired experiments for each group. In the basal period, peripheral insulin levels (PER, 49.9 ± 4.6 pmol/L; POR, 56.4 ± 11.2; ½ PER, 49.8 ± 9.6; Fig 2) and calculated hepatic sinusoidal insulin levels (Table 1) were slightly but not significantly higher in POR versus PER or ½ PER. During the clamp, peripheral insulin levels (Fig 2) were minimally increased with all treatments and were greater with PER versus POR or ½ PER ($P < .001$). There was no difference in peripheral insulin levels between POR and ½ PER, as per the experimental design. During the clamp period, the calculated hepatic sinusoidal insulin levels (Table 1) were greatest with POR ($P < .001$ *v* PER or ½ PER), as expected. Plasma glucose levels were maintained constant at moderate hyperglycemia in all treatments (Fig 2). The glucose infusion rate (GINF) necessary to maintain glycemia constant (Fig 2) was greater in

Table 1. Hepatic Sinusoidal Insulin Levels (mean ± SEM) during PER, POR, or 1/2 PER Insulin Infusion

Parameter	PER	POR	1/2 PER
Estimated hepatic insulin (pmol/L) by Fick's method			
Basal	104.2 ± 10.9	123.3 ± 20.6	104.5 ± 19.9
Clamp	132.0 ± 13.2	163.0 ± 21.3*	119.0 ± 20.3
Estimated hepatic insulin (pmol/L) by De Feo's method			
Basal	100.5 ± 9.3	113.2 ± 22.5	100.1 ± 19.2
Clamp	129.3 ± 9.8	148.1 ± 23.6*	114.6 ± 19.3

NOTE. Basal refers to −40 to 0 minutes; clamp refers to 90 to 180 minutes.

*$P < .001$, POR *v* PER or 1/2 PER.

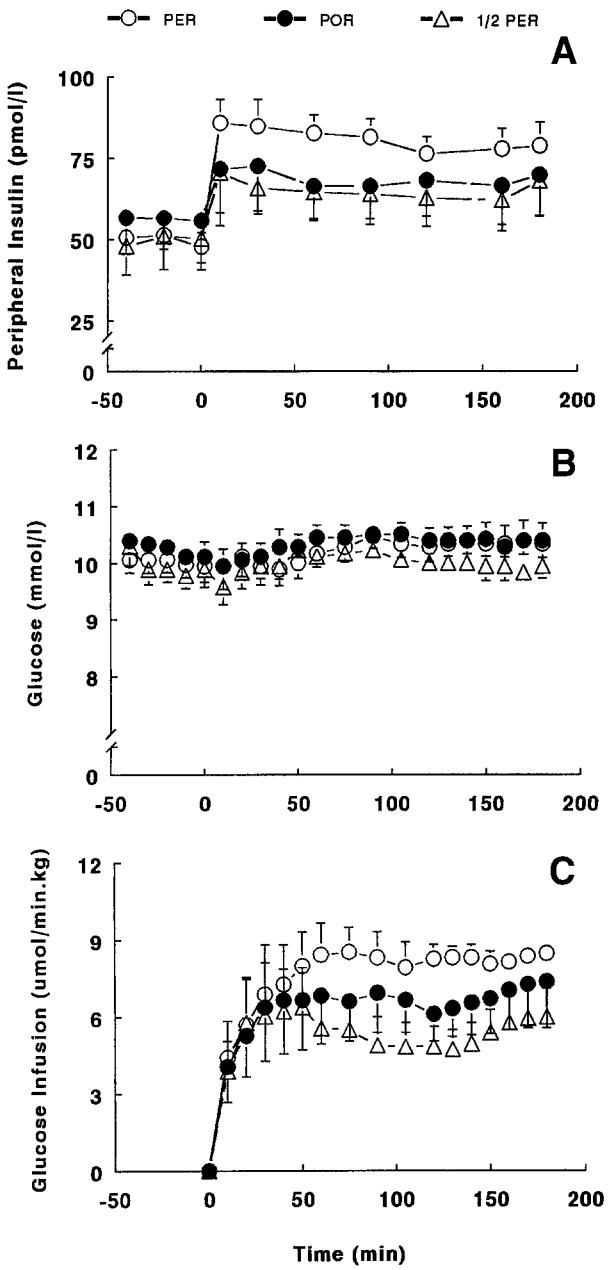

Fig 2. Peripheral insulin levels (A), plasma glucose levels (B), and glucose infusion rates (C) in the 3 experimental groups. Experiments were performed in depancreatized dogs which were maintained at moderate hyperglycemia (~10 mmol/L) by subbasal insulin infusions (see Fig 1). Measurements were taken before (basal) and during additional equidose peripheral (○) or portal (●) insulin infusions of 0.75 pmol/kg · min or half-dose peripheral (△) insulin infusion of 0.375 pmol/kg · min. A glucose clamp was maintained by infusing a mixture of labeled and unlabeled glucose, as described in the text. Values are presented as the mean ± SEM from 6 paired experiments per group.

PER versus POR or ½ PER ($P < .001$). There was also a significant difference in the GINF between POR and ½ PER ($P < .01$).

Specific activity remained constant in all treatments (Fig 3). Basal glucose utilization (PER, 14.8 ± 1.6 μmol/kg · min; POR, 16.6 ± 1.2; ½ PER, 16.0 ± 1.0) was lowest in PER, and the difference between POR and PER was significant ($P < .05$). During the clamp, glucose utilization increased minimally in PER but did not increase in POR or ½ PER (Fig 3). When expressed as an absolute value, glucose utilization during the clamp was 16.0 ± 1.0 μmol/kg · min in PER, 16.8 ± 1.3 in POR, and 15.5 ± 0.8 in ½ PER. In ½ PER, glucose utilization during the clamp was significantly lower than in POR ($P < .001$).

GP in the basal period was lower with PER (14.1 ± 1.1 μmol/kg · min) versus POR (15.4 ± 0.9, $P < .05$) or ½ PER (15.6 ± 0.8, $P < .01$). During the clamp, GP decreased to a lower level ($P < .001$) with PER (7.8 ± 0.8 μmol/kg · min) versus POR (10.0 ± 1.1) or ½ PER (9.9 ± 0.8). The steady-state (90 to 180 minutes) percentage suppression of GP (Fig 3) in PER was 45.4% ± 4.0%, significantly more than for POR or ½ PER (35.3% ± 6.8% and 35.6% ± 6.3%, $P < .001$ and $P < .01$, respectively). There was no difference between the suppression of GP obtained with POR or ½ PER.

Glucagon levels were lower with POR and PER than with ½ PER throughout the experiment; however, significant differences ($P < .001$, ½ PER *v* POR or PER) were only observed during the clamp period (Fig 4). The percentage suppression of glucagon was greater with PER versus POR or ½ PER ($P < .001$). Basal NEFA levels were lower with PER than with POR ($P < .01$). NEFA levels during the clamp were also lower with PER ($P < .001$ *v* POR and $P < .01$ *v* ½ PER). The percentage decrease in NEFA levels from basal was not significantly different in the three groups (Fig 4). Lactate and alanine did not change significantly from basal, whereas glycerol declined with all treatments (Table 2). There was no significant difference in lactate, alanine, and glycerol levels between the three treatments.

DISCUSSION

In this study in depancreatized dogs under conditions of moderate hyperglycemia and insulin levels in the fasting range, the suppression of GP was proportional to peripheral insulin levels, not hepatic sinusoidal insulin levels. These results are consistent with those obtained in the same animal model using higher insulin infusion rates. In contrast to the results obtained using the same insulin infusion rates in nondiabetic dogs,[15] no hepatic effect of insulin on GP was observed, which suggests that in our depancreatized dog model the regulation of GP is more dependent on peripheral insulin than in nondiabetic dogs.

The GINF with the PER treatment was significantly greater than with POR or ½ PER, consistent with the greater suppression of GP and the greater stimulation of glucose utilization. The somewhat greater glucose requirement with POR versus ½ PER was not due to a difference in GP since GP rates were equal, but was likely due the small difference in glucose utilization. The reason for the latter difference is unclear, but it might be related, at least in part, to greater hepatic glucose uptake in POR versus ½ PER because of higher hepatic insulin levels and/or to a delayed effect of the nonsignificantly greater

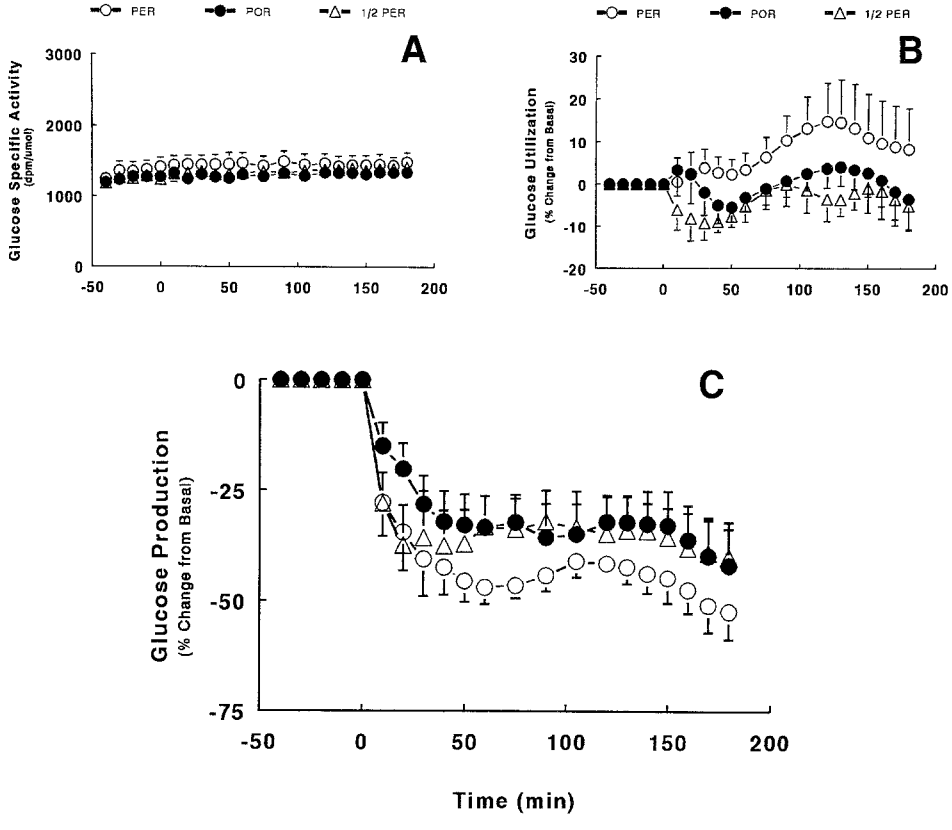

Fig 3. Glucose specific activity (A), glucose utilization (B), and glucose production (C) in the 3 experimental groups. Experimental design is outlined in the legend to Fig 2. Values are presented as the mean ± SEM.

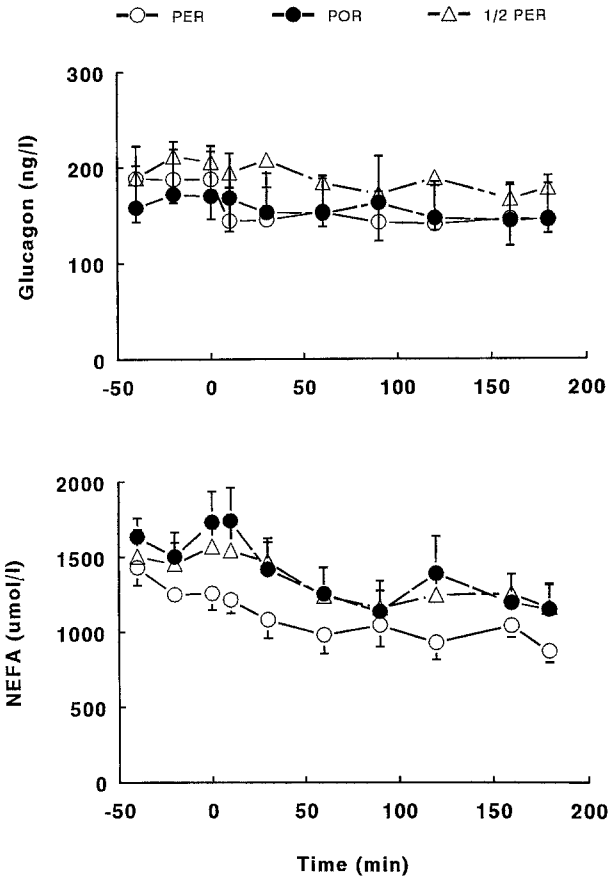

Fig 4. Glucagon (A) and NEFA levels (B) in the 3 experimental groups. Experimental design is outlined in the legend to Fig 2. Values are presented as the mean ± SEM.

insulinization in the POR versus ½ PER group during the preclamp period.

As in our previous studies in normal dogs, the extent of suppression of GP observed in the presence of small increases in insulin was greater than expected in all groups. NEFA and glucagon decreased in all groups, and since a difference in hepatic insulinization did not affect GP, it is possible that the peripheral effect of insulin was sufficient to account for the full extent of GP suppression, although we cannot exclude the possibility that part of the GP suppression was due to a hepatic effect of insulin that saturates at lower hepatic insulin concentrations than those achieved in ½ PER. Also, we cannot exclude the possibility that part of the GP suppression observed in all groups was not due to insulin, but to an overestimation of glucose requirements during the clamp. Deliberate increases in the GINF have been shown to decrease GP in the absence of detectable changes in plasma glucose.[37] However, differences in the GINF between groups cannot account for the failure of POR to suppress GP more than ½ PER in the present study, since the GINF was higher rather than lower in the POR group.

Table 2. Plasma Metabolite Levels (mean ± SEM) During PER, POR, or 1/2 PER Insulin Infusion

Metabolite	PER	POR	1/2 PER
Lactate (μmol/L)			
Basal	617.8 ± 58.0	586.7 ± 36.5	609.5 ± 72.2
Clamp	610.7 ± 102.4	513.3 ± 40.4†	634.7 ± 90.5
Alanine (μmol/L)			
Basal	556.5 ± 46.6	389.8 ± 47.9	474.3 ± 65.8
Clamp	565.0 ± 112.2	383.4 ± 36.8	527.2 ± 71.5†
Glycerol (μmol/L)			
Basal	129.8 ± 10.0	145.3 ± 7.7	142.0 ± 8.3
Clamp	98.4 ± 7.8*	107.8 ± 4.9*	122.7 ± 8.3†

NOTE. Basal refers to −40 to 0 minutes; clamp refers to 90 to 180 minutes.

*$P < .001$, clamp *v* basal.

†$P < .05$, clamp *v* basal.

Peripheral insulin suppresses GP by limiting the availability of NEFA.[11,12,38] In the present study, there was a significant difference in NEFA during the clamp period, although this difference appeared to be due, at least in part, to differences in the basal levels. Even a small difference in NEFA might have a greater impact on GP in diabetes because gluconeogenesis, which is dependent on NEFA for energy supply, accounts for a greater percentage of GP than under nondiabetic conditions. A limitation in the availability of gluconeogenic precursors may contribute to insulin's peripheral suppression of GP, although quantitatively, this contribution appears minimal.[12,13,19] Significant differences in alanine and glycerol were found in our previous study at high insulin levels, but not in the present study.

It is also possible that the dependence of GP suppression on peripheral insulin is more accentuated in depancreatized versus normal dogs, because of a greater effect of exogenous insulin on glucagon secretion in depancreatized dogs. When endogenous insulin secretion is present as in our nondiabetic dogs (who did not receive somatostatin), pancreatic glucagon suppression may be mainly dependent on intraislet insulin. In contrast, when endogenous insulin secretion is absent, as in depancreatized dogs, the suppression of glucagon is only dependent on exogenous insulin reaching the pancreatic α cell or the oxyntic cell at arterial (systemic) concentrations. In the present study, as well as in our previous study in depancreatized dogs, there was a trend for glucagon concentrations to decrease more with PER versus POR or ½ PER. In contrast, in nondiabetic dogs no such trend was found.[15]

In addition, it has been suggested that renal GP accounts for up to 20% of total GP.[39-41] In diabetic dogs, a larger portion of GP appears to be produced by the kidney than in nondiabetic dogs.[42] Since the kidney is exposed to the peripheral insulin concentrations, renal GP could account for part of the peripheral effect of insulin on GP and its accentuation in diabetes.

The previously discussed possibilities account for a relative decrease of the hepatic versus peripheral effect of insulin under diabetic compared with nondiabetic conditions. However, two lines of evidence suggest that the hepatic effect of insulin is not only relatively but also absolutely reduced under diabetic conditions: (1) our inability to detect a differential inhibition of GP by hepatic insulin at matched peripheral insulin concentrations in diabetic dogs, whereas hepatic insulin inhibited GP in normal dogs, and (2) a greater impairment of GP suppression

with POR versus PER or ½ PER in diabetic versus normal dogs.[15]

Our inability to detect a hepatic effect of insulin does not exclude the possibility that a small effect was present but we did not have the power to detect it. We had a 95% power to detect a 1.4-μmol/kg · min difference in GP. Since differences in GP between POR and ½ PER were significant in studies with similar power performed using the same insulin infusion rates in nondiabetic dogs,[15] it appears that the hepatic effect of insulin, whether present or not, was reduced in diabetic versus normal dogs.

One factor that could diminish the hepatic effect of insulin is laminar flow in the portal circulation, resulting in nonhomogeneous insulin delivery and therefore regions of hepatic underinsulinization. However, several observations indicate that this factor did not have a major influence on our results: (1) the calculated first-pass hepatic insulin extraction was about 50%, which indicates that insulin did not bypass the liver; (2) the surgical preparation in the present study was the same as in nondiabetic dogs, in which the hepatic effect of insulin could be detected[15]; and (3) the results in nondiabetic dogs were similar to those in humans, where endogenous insulin secretion was stimulated with tolbutamide (resulting in physiological mixing of insulin in the portal vein).[16]

There may be several reasons for a reduction of the hepatic effect of insulin in diabetic dogs. First, hepatic insulin resistance may have decreased the direct action of insulin in the liver while leaving the peripheral action of insulin on glucagon suppression and/or lipolysis relatively unaffected. Insulin resistance is found in diabetic depancreatized dogs as in type 1 diabetes. This form of insulin resistance is presumably secondary to the metabolic derangement of diabetes, including chronic hyperglycemia. It might also be argued that the initial 1 to 2 hours of marked hyperinsulinemia due to the portal insulin infusion used in the preclamp period may have acutely induced hepatic insulin resistance. However, results from prolonged glucose clamps[43-47] suggest that short-term hyperinsulinemia (up to 7 hours), unlike prolonged hyperinsulinemia,[48,49] does not downregulate insulin action.

Another reason for the apparent absence of the direct effect of insulin on GP in depancreatized dogs is the hyperglycemic level per se in our experimental conditions, independent of the effect of hyperglycemia to induce hepatic insulin resistance in a chronic setting. Our diabetic dogs were studied at hyperglycemia, whereas the normal dogs were studied at euglycemia. Indeed, our preliminary data suggest that acute correction of hyperglycemia, obtained by increasing the rate of preclamp portal insulin infusion, restores the hepatic effect of insulin on GP in depancreatized dogs.[50] Hyperglycemia can inhibit glycogenolysis more than gluconeogenesis.[51] Since the direct effect of insulin on GP mainly consists of inhibition of glycogenolysis,[13,14] this effect might be diminished under hyperglycemic conditions, when glycogenolysis is suppressed. Glycogenolysis in diabetic dogs may also be limited by decreased glycogen stores.

In conclusion, we have shown that in moderately hyperglycemic depancreatized dogs, insulin regulation of GP is predominantly a peripheral effect of insulin under conditions of both basal (present study) and high physiological insulin concentrations.[19] In contrast to our data in nondiabetic dogs[15] and humans,[16] we found no evidence for a direct hepatic effect of insulin on GP.

Our results have important clinical implications, since they suggest that in insulin-treated diabetes under conditions of moderate hyperglycemia, a portal-peripheral insulin gradient may not be a crucial factor in the regulation of GP. However, the present findings should be interpreted in the context of our model and experimental conditions. It is likely that in a better-controlled or less insulin-resistant model of diabetes, direct effects of insulin on GP can be unmasked. In addition, our findings refer to the acute effect of insulin on GP. It is possible that higher hepatic versus peripheral insulin levels are necessary in the long-term regulation of GP.[52-54]

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REFERENCES

1. Greenway CV, Stark RD: Hepatic vascular bed. Pediatr Res 51:23-65, 1971

2. Stevenson RW, Parsons JA, Alberti KGMM: Effect of intraportal and peripheral insulin on glucose turnover and recycling in diabetic dogs. Am J Physiol 244:E190-E195, 1983

3. Ishida T, Chap Z, Chou J, et al: Effects of portal and peripheral venous insulin infusion on glucose production and utilization in depancreatized dogs. Diabetes 33:984-990, 1984

4. Rizza RA, Westland RE, Hall LD, et al: Effect of peripheral versus portal venous administration of insulin on postpandrial hyperglycemia and glucose turnover in alloxan-diabetic dogs. Mayo Clin Proc 56:434-438, 1981

5. Fischer U, Rizza RA, Hall LD, et al: Comparison of peripheral and portal venous insulin administration on postprandial metabolic responses in alloxan-diabetic dogs. Effects of identical preprogrammed complex insulin infusion waveforms. Diabetes 31:579-584, 1992

6. Freyse EJ, Rebrin K, Schneider T, et al: Increasing urea synthesis in insulin-dependent diabetic dogs maintained normoglycemic: Effect of portal insulin administration and food protein content. Diabetes 45:667-674, 1996

7. Freyse EJ, Fisher V, Albrecht G, et al: The effect of prehepatic insulin administration on alanine flux rates in diabetic dogs. Diabetologia 30:402-408, 1987

8. Monti LD, Piatti PM, Home PD, et al: The effect of intraperitoneal insulin delivery on carbohydrate metabolism in type 1 (insulin-dependent) diabetic patients. Diabetes Res Clin Pract 15:237-244, 1992

9. Ader M, Bergman RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. Am J Physiol 245:E1020-E1032, 1990

10. Rebrin K, Steil GM, Getty L, et al: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. Diabetes 44:1038-1045, 1995

11. Rebrin K, Steil GM, Mittelman SD, et al: Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. J Clin Invest 98:741-749, 1996

12. Sindelar DK, Chu CA, Rohlie M, et al: The role of fatty acids in

mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. Diabetes 46:187-196, 1997

13. Sindelar DK, Balcom JH, Chu CA, et al: A comparison of the effects of selective increases in peripheral or portal insulin on hepatic glucose production in the conscious dog. Diabetes 45:1594-1604, 1996

14. Sindelar DK, Chu CA, Neal DW, et al: Interaction of equal increments in arterial and portal vein insulin on hepatic glucose production in the dog. Am J Physiol 273:E972-E980, 1997

15. McCall RH, Wiesenthal SR, Shi ZQ, et al: Insulin acutely suppresses glucose production by both peripheral and hepatic effect in normal dogs. Am J Physiol 274:E346-E356, 1998

16. Lewis GF, Zinman B, Groenewoud Y, et al: Hepatic glucose production is regulated both by direct hepatic and extrahepatic effects of insulin in humans. Diabetes 45:454-462, 1996

17. Ducimetiere P, Eschwege E, Popoz L, et al: Relationship of plasma insulin levels to the incidence of myocardium infarction and coronary heart disease mortality in a middle aged population. Diabetologia 19:205-210, 1980

18. Cambien F, Warnet JM, Eschwege E, et al: Body mass, blood pressure, glucose, and lipids: Does plasma insulin explain their relationships? Arteriosclerosis 7:197-202, 1987

19. Giacca A, Fisher S, Shi ZQ, et al: Importance of peripheral insulin levels for insulin-induced suppression of glucose production in depancreatized dogs. J Clin Invest 90:1769-1777, 1992

20. Hatton TW, Yip CC, Vranic M: Biosynthesis of glucagon (IRG3500) in canine gastric mucosa. Diabetes 34:38-46, 1985

21. Doi K, Prentki M, Yip C, et al: Identical biological effects of pancreatic glucagon and a purified moiety of canine gastric glucagon. J Clin Invest 63:525-531, 1979

22. Vranic M, Kawamori R, Pek S, et al: The essentiality of insulin and the role of glucagon in regulating glucose utilization and production during strenuous exercise in dogs. J Clin Invest 57:245-256, 1976

23. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

24. Finegood DT, Bergman RN, Vranic M: Modelling error and apparent isotope discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. Diabetes 37: 1025-1034, 1988

25. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic euglycemic glucose clamp: Comparison of labelled and unlabelled glucose infusate. Diabetes 36:914-924, 1987

26. Schwenk WF, Butler PC, Haymond MW, et al: Underestimation of glucose turnover corrected with high-performance liquid chromatography purification of [6-^{3}H] glucose. Am J Physiol 258:E228-E233, 1990

27. Kadish AH, Sternberg JC: Determination of urine glucose by measurement of rate of oxygen consumption. Diabetes 18:467-470, 1969

28. Herbert V, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

29. Faloona GR, Unger RH: Glucagon, in Jaffe BM, Berman HR (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic, 1974, pp 317-330

30. Ho RJ: Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. Anal Biochem 36:105-113, 1970

31. Lloyd B, Burrin J, Smythe P, et al: Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol, and 3-OH-butyrate. Clin Chem 24:1724-1729, 1978

32. Bjorkman O, Miles P, Wasserman D, et al: Regulation of glucose turnover during exercise in pancreatectomized, totally insulin deficient dogs: Effects of beta-adrenergic blockade. J Clin Invest 81:1759-1767, 1988

33. Finegood DT, Bergman RN: Optimal segments: A method for smoothing tracer data to calculate metabolic fluxes. Am J Physiol 244:E472-E479, 1983

34. Bradley DC, Steil GM, Bergman RN: Quantitation of measurement error with optimal segments: Basis for adaptive time course smoothing. Am J Physiol 264:E902-E911, 1993

35. deBodo RC, Steele R, Altszuler N, et al: On the hormonal regulation of carbohydrate metabolism: Studies with ^{14}C glucose. Recent Prog Horm Res 19:445-488, 1963

36. De Feo P, Perriello G, De Cosmo S, et al: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. Diabetes 35:563-569, 1986

37. Sheu WH-H, Hollenbeck CB, Wu M-S, et al: Effect of difference in glucose infusion rate on quantification of hepatic glucose production. J Clin Endocrinol Metab 70:1354-1360, 1990

38. Lewis GF, Vranic M, Harley P, et al: Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes 46:1111-1119, 1997

39. McGuinness OP, Fugiwara T, Murrell S, et al: Impact of chronic stress hormone infusion on hepatic carbohydrate metabolism in the conscious dog. Am J Physiol 265:E314-E322, 1993

40. Stumvoll M, Chintalapudi U, Perriello G, et al: Uptake and release of glucose by the human kidney. J Clin Invest 96:2528-2533, 1995

41. Cersosimo E, Judd RL, Miles JM: Insulin regulation of renal glucose metabolism in conscious dogs. J Clin Invest 93:2584-2589, 1994

42. Stevenson RW, Williams PE, Cherrington AD: Role of glucagon suppression on gluconeogenesis during insulin treatment of the conscious diabetic dog. Diabetologia 30:782-790, 1987

43. Dauber L, Greenfield MS, Schulz B, et al: Enhanced glucose utilization during prolonged glucose clamp studies. Diabetes 30:829-835, 1981

44. Boden G, Jadali F, White J, et al: Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 88:960-966, 1991

45. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid–induced inhibition of glucose uptake. J Clin Invest 93:2438-2446, 1994

46. Rossetti L, Hawkins M, Chen W, et al: In vivo glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. J Clin Invest 96:132-140, 1995

47. Baron AD, Zhu J-S, Zhu J-H, et al: Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle: Implications for glucose toxicity. J Clin Invest 96:2792-2801, 1995

48. Rizza RA, Mandarino LJ, Genest J, et al: Production of insulin resistance by hyperinsulinaemia in man. Diabetologia 28:70-75, 1985

49. Marangou AG, Weber KM, Boston RC, et al: Metabolic consequences of prolonged hyperinsulinemia in humans. Diabetes 35:1383-1389, 1986

50. Gupta N, Sandhu H, Goh T, et al: Euglycemia acutely restores the direct hepatic effect of insulin on glucose production (GP) in depancreatized dogs. Diabetes 47:A41, 1998 (suppl 1, abstr)

51. Rossetti L, Giaccari A, Barzilai N, et al: Mechanism by which hyperglycemia inhibits hepatic glucose production in conscious rats. J Clin Invest 92:1126-1134, 1993

52. Goriya Y, Bahoric A, Marliss EB, et al: Glycemic regulation using a programmed insulin delivery device. III. Long-term studies on diabetic dogs. Diabetologia 19:452-457, 1980

53. Goriya Y, Bahoric A, Marliss EB, et al: The metabolic and hormonal responses to a mixed meal in unrestrained pancreatectomised dogs chronically treated by portal or peripheral insulin infusion. Diabetologia 21:58-64, 1981

54. Luzi L, Hering BJ, Socci C, et al: Metabolic effects of successful intraportal islet transplantation in insulin-dependent diabetes mellitus. J Clin Invest 97:2611-2618, 1996