

# Resistance to Insulin's Acute Direct Hepatic Effect in Suppressing Steady-State Glucose Production in Individuals With Type 2 Diabetes

Gary F. Lewis, André Carpentier, Mladen Vranic, and Adria Giacca

We and others have shown that insulin acutely suppresses glucose production in fasting nondiabetic humans and dogs, by both a direct hepatic effect and an indirect (extrahepatic) effect, and in diabetic dogs by an indirect effect alone. In type 2 diabetes, there is resistance to insulin's ability to suppress hepatic glucose production, but it has not previously been determined whether the resistance is primarily at the level of the hepatocyte or the peripheral tissues. To determine whether the diabetic state reduces the direct effect of insulin in humans, we studied nine patients with untreated type 2 diabetes who underwent three studies each, 4–6 weeks apart. 1) Portal study (POR): intravenous tolbutamide was infused for 3 h with calculation of pancreatic insulin secretion from peripheral plasma C-peptide. 2) Peripheral study (PER): equidose insulin was infused by peripheral vein. 3) Half-dose peripheral insulin study (1/2 PER): matched peripheral insulin levels with study 1. In all studies, glucose was clamped at euglycemia, glucose turnover was measured with the constant specific activity method, and 3-[<sup>3</sup>H]glucose was purified by high-performance liquid chromatography. Peripheral insulin was lower in POR versus PER but slightly higher in POR versus 1/2 PER, although most of the difference could be accounted for by higher proinsulin levels in POR (stimulated by tolbutamide). Calculated portal insulin was ~1.3-fold higher in POR versus PER and ~2.2-fold higher in POR versus 1/2 PER. In the final 30 min of the clamp, glucose production reached a lower steady-state level in PER than in POR ( $4.0 \pm 0.4$  vs.  $5.3 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$ ), despite the higher hepatic insulin level in POR. In contrast with our studies in nondiabetic individuals, glucose production was not more suppressed at steady state in POR versus 1/2 PER ( $5.3 \pm 0.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), despite much higher hepatic insulin levels in POR. In conclusion, this is the first study in patients with type 2 diabetes to characterize insulin resistance to the acute direct suppressive effect of insulin on hepatic glucose production. *Diabetes* 48:570–576, 1999

From the Departments of Medicine (G.F.L., A.C., M.V., A.G.) and Physiology (M.V., A.G.), University of Toronto, Toronto, Ontario, Canada.

Address correspondence and reprint requests to Dr. Gary Lewis, The Toronto Hospital, General Division, 200 Elizabeth St., Room EN 11-229, Toronto, Ontario, Canada M5G 2C4. E-mail: glewis@torhosp.toronto.on.ca.

Received for publication 12 June 1998 and accepted in revised form 13 November 1998.

FFA, free fatty acid; GP, glucose production; GU, glucose utilization; HPLC, high-performance liquid chromatography; PER, peripheral insulin study; 1/2 PER, half-dose peripheral insulin study; POR, portal insulin study;  $R_g$ , rate of glucose production;  $R_d$ , rate of glucose utilization; SA, specific activity.

Pancreatic hormones are normally secreted into the portal venous system, and ~40–60% of insulin is cleared on the first pass through the liver (1,2). Hepatic insulin clearance followed by dilution in the larger systemic circulation leads to a physiologic portal-peripheral (systemic) insulin concentration gradient, with portal and hepatic insulin levels exceeding those in the peripheral venous circulation (3). Insulin acutely suppresses hepatic glucose production (GP), and the acute suppression is mediated partly by a direct hepatic effect and partly by an indirect or extrahepatic effect of insulin (4–7). There is now ample evidence that insulin's suppressive effect on peripheral tissue lipolysis, with reduction in plasma free fatty acids (FFAs), is an important signal mediating the indirect effect of insulin on hepatic GP (4,8–13). Glucagon potentiates the direct effect of insulin (6,14,15), and we have recently demonstrated that the insulin-induced suppression of glucagon and FFAs has additive or cooperative effects in mediating the acute extrahepatic effect of insulin on GP (12).

Higher hepatic insulin concentrations appear to be necessary to rapidly inhibit hepatic glucose production during the transition from the fasted to the fed state, and slightly delayed feedback control of hepatic glucose production by insulin-mediated processes in peripheral tissues provides a second order of regulation, one more responsive to the energy needs of the organism. Portal insulin delivery with ~50% hepatic insulin extraction provides the liver with appropriately high insulin concentrations to rapidly suppress glucose production while limiting the degree of potentially deleterious peripheral hyperinsulinemia. Peripheral control of hepatic glucose production, on the other hand, permits the response of hepatic fuel synthesis to be coordinated with the metabolic effects of peripheral insulin, sensing decreased peripheral tissue energy needs when insulin, glucose, and fats are plentiful, such as occurs in the postprandial state. This complex system of feedback control provides a smooth transition from the fasted to the fed state.

The balance between the direct and indirect effects of insulin in suppressing GP depends on the relative exposure of liver and extrahepatic tissues to insulin and the sensitivity of the tissues to insulin. In patients with type 1 or type 2 diabetes, insulin treatment is generally administered by subcutaneous injection, thus abolishing the normal portal-periph-

TABLE 1  
Clinical characteristics of subjects and mean basal values

Subject	Sex (M/F)	Age (years)	Duration of diabetes (years)	Glucose (mmol/l)	HbA <sub>1c</sub> (%)	BMI (kg/m <sup>2</sup> )	Insulin (pmol/l)	Glucagon (pg/ml)	FFAs (mmol/l)	R <sub>a</sub> (μmol · kg <sup>-1</sup> · min <sup>-1</sup> )
1	M	63	3	7.5	8.6	33.1	80.1	74.5	0.64	10.3
2	F	44	5	8.5	6.9	24.7	42.0	63.4	0.51	10.6
3	M	53	4	10.9	12.3	33.1	58.2	64.8	0.55	13.8
4	M	55	3	8.2	7.9	28.9	36.0	61.0	0.66	12.1
5	M	63	6	12.1	10.3	27.9	32.4	43.2	0.58	13.9
6	M	42	5	9.5	9.9	26.0	99.1	103.0	0.67	9.7
7	F	46	2	7.2	6.6	41.2	54.3	97.8	0.77	8.3
8	M	53	4	7.1	6.7	31.4	36.6	78.7	0.57	10.4
9	F	58	2	7.0	7.1	23.7	48.2	57.9	0.71	9.9
Total	6 M/3 F	53 ± 3	3.8 ± 0.5	8.7 ± 0.6	8.5 ± 0.7	30.0 ± 1.8	54.1 ± 7.5	71.6 ± 6.4	0.63 ± 0.02	11.0 ± 0.6

Data are means ± SE for each subject for basal values (-40 to 0 min) for all three studies (portal, peripheral, and half-dose peripheral).

eral insulin concentration gradient, and glycemia is controlled at the expense of higher peripheral insulin concentrations (16). Both in vitro (17–19) and in vivo (20–22) experiments suggest that chronically increased peripheral insulin concentrations can cause insulin resistance, not only aggravating existing insulin resistance but also potentially causing a number of other adverse metabolic consequences.

In type 2 diabetes, there is well-described hepatic resistance to the suppressive effect of insulin on glucose production (23,24), but previous studies have not examined whether this resistance to insulin's effect is at the level of the hepatocyte or the peripheral tissues, the latter with consequent indirect hepatic effects. In contrast with our studies in nondiabetic humans (5) and dogs (25), our earlier studies in depancreatized dogs (26) failed to demonstrate a direct hepatic effect of insulin in suppressing GP (26). The mechanism accounting for this difference between diabetic and nondiabetic dogs is not known, but it appears to indicate that there is resistance to the acute direct suppressive effect of insulin at the hepatocyte level in diabetic dogs, perhaps due to the effects of hyperglycemia per se. This concept is supported by our recent observations that restoration of euglycemia acutely restores the direct hepatic effect of insulin on GP in depancreatized dogs (27). To determine whether the diabetic state also reduces the direct effect of insulin in humans, in the present study we examined the acute effects of portal versus peripheral insulin delivery on GP in patients with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

**Subjects.** The clinical characteristics of the nine study subjects with untreated type 2 diabetes are outlined in Table 1. No subject had a history of systemic illness other than diabetes, and none had taken any diabetes medication or medication known to affect carbohydrate metabolism during the 6-month period preceding the study or during the study. Informed written consent was obtained from all participants in accordance with the guidelines of the Human Subjects Review Committee of the Toronto Hospital, University of Toronto. Permission was granted by the Health Protection Branch, Health and Welfare Canada, for the use of intravenous tolbutamide (control number 034076, G.F.L.).

**Experimental protocol.** All subjects were studied on three occasions each, with a minimum of 4 weeks and a maximum of 6 weeks between studies. The order of the studies was not randomized, since the tolbutamide infusion study (portal study) had to be performed first in all subjects to calculate the insulin infusion rates for the second and third studies. The subjects were instructed not to make major changes to their diet, exercise habits, or alcohol intake between studies. In the first study, hyperinsulinemia was induced by an intravenous tolbutamide infusion

(the portal study [POR]). In the second study, hyperinsulinemia was induced by an exogenous insulin infusion (the full-rate peripheral insulin study [PER]). In the third study, exogenous insulin was infused at half the rate infused in PER (the half-rate peripheral insulin study [1/2 PER]).

**Portal studies.** Subjects were admitted to the Metabolic Investigation Unit of the Toronto Hospital following a 12-h overnight fast and did not eat until completion of the study that afternoon. At ~8:00 A.M. (time -150 min) a primed ( $3.3 \times 10^6$  dpm) continuous infusion ( $0.33 \times 10^6$  dpm/min) of 3-[<sup>3</sup>H]glucose (New England Nuclear, Boston, MA) was started and maintained throughout the study. The tracer had been submitted to the high-performance liquid chromatography (HPLC)-purification procedure (28). After 110 min, five samples of arterialized venous blood were drawn every 10 min for basal determinations from an intravenous catheter placed in a dorsal hand vein of the opposite arm, which was maintained in a warming device. At time 0 min, tolbutamide sodium, USP (Upjohn, Kalamazoo, MI), 3 g in 250 ml normal saline, was infused into a peripheral arm vein at a rate of 1 g/h for the 1st hour, 800 mg/h for the 2nd hour, and 600 mg/h for the 3rd hour. This dose was empirically determined in earlier studies to produce sustained and steady rates of pancreatic insulin secretion in nondiabetic individuals (29). The mean steady rate of pancreatic insulin secretion between 60 and 180 min in response to tolbutamide was used to determine the exogenous insulin infusion rate needed for the second study. Blood samples were drawn for glucose, 3-[<sup>3</sup>H]glucose specific activity (SA), insulin, glucagon, C-peptide, proinsulin, and FFAs at baseline and at regular intervals throughout the study. Samples for measurement of FFAs were drawn into chilled EDTA tubes on ice containing 0.4 mmol/l of the lipase inhibitor APBA (m-aminophenylboronic acid; Sigma, St. Louis, MO) (30). Plasma glucose levels were measured every 5 min during the tolbutamide infusion. The values were used to adjust the rate of a 20% dextrose infusion to maintain constant isoglycemia (that is, plasma glucose level was maintained at the patient's fasting level, determined during the basal period of each study). An aliquot of 3-[<sup>3</sup>H]glucose was added to the 20% dextrose infusate (0.388 μCi/kg 3-[<sup>3</sup>H]glucose added to 500 ml dextrose solution) to minimize the decline in glucose SA during the clamp (hot glucose infusion [Ginf] method) (31,32).

Tolbutamide was discontinued after 3 h, and the subjects were permitted to eat, thus ending the active phase of the study. Since tolbutamide has a prolonged action, the rate of the 20% dextrose infusion was gradually reduced overnight, maintaining iso- or euglycemia at all times according to half-hourly blood glucose measurements drawn through a sampling intravenous catheter. In most cases, the dextrose infusion was discontinued within 6 h of stopping the tolbutamide.

**Full-rate peripheral insulin study.** The study using an exogenous insulin infusion was performed 4–6 weeks later using crystalline human insulin (Novo Nordisk Canada, Toronto, Canada) infused into a peripheral vein between 0 and 180 min, following a 150-min basal equilibration period. Plasma glucose levels were maintained at isoglycemia for each patient as described above. The rate of infusion of exogenous insulin was matched in each individual to the calculated mean steady rate of pancreatic insulin secretion between 60 and 180 min of the earlier tolbutamide infusion. Pancreatic insulin secretion had been calculated from peripheral plasma C-peptide levels by deconvolution using a two-compartment mathematical model for C-peptide distribution and metabolism as previously described (33). The software program for calculation of insulin secretion was provided by Drs. K. Polonsky and J. Sturis, University of Chicago, Chicago. The use of standard parameters for C-peptide clearance and distribution has been shown to result in insulin secre-

tion rates that differ in each subject by only 10–12% from those obtained with individual parameters, and there is no systematic over- or underestimation of insulin secretion (33). Over the 1st hour of the infusion (0–60 min), the insulin infusion rate was increased in increments of 25% of the calculated maximal rate every 15 min, to mimic as closely as possible the gradual increase in insulin secretion seen in the earlier portal insulin study.

**Half-rate peripheral insulin infusion study.** This study was performed 4–6 weeks after the full-rate peripheral insulin infusion study described above and was identical to that study, except that insulin was infused at half the rate in an attempt to match peripheral venous insulin concentrations with those in the portal insulin study.

**Calculations.** The SA of the infusate was calculated as previously described (5). Briefly, calculations were based on estimation of the parameters of the formula of Finegood et al. (31), modified to allow for incomplete suppression of GP. GP was calculated as the endogenous rate of appearance measured with 3-[<sup>3</sup>H]glucose; glucose utilization (GU) as the rate of disappearance measured with 3-[<sup>3</sup>H]glucose ( $R_d$ ). For glucose turnover calculations, a modified one-compartment model (31) was used to account for the exogenously infused mixture of labeled and unlabeled glucose. Data were smoothed with the optimal segments routine (34) using the optimal error algorithm (35). With the hot Ginf method, the monocompartment assumption becomes minor, because the non-steady-state part of the Steele's equation is close to zero.  $R_d$  corresponded to GU, and plasma clearance rate of glucose ( $R_d$ /glycemia) corresponded to glucose metabolic clearance rate (MCR), since plasma glucose levels were below the renal threshold for glycosuria.

Portal insulin levels were calculated according to the method of De Feo et al. (36):

$$\frac{PI_t}{ISR_t} = \frac{AI_t + [PI_0 - AI_0]ISR_0}{ISR_0}$$

$PI_t$ ,  $AI_t$ , and  $ISR_t$  are the portal venous insulin concentration, arterial (peripheral) plasma insulin concentration, and insulin secretion rate, respectively, at time  $t$ , and  $PI_0$ ,  $AI_0$ , and  $ISR_0$  are those at baseline.  $ISR_0$  was taken as the average of the basal insulin secretion rates determined in the three experiments.  $PI_0$  was assumed to be  $2.4 \times AI_0$  (36). Hepatic insulin levels were calculated assuming a 72% vascularization of the liver by the portal vein and 28% by the hepatic artery (37).

**Laboratory methods.** Glucose was assayed enzymatically at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Insulin was measured by radioimmunoassay using a double-antibody separation method (kit supplied by Pharmacia Diagnostic, Uppsala, Sweden). C-peptide was measured by radioimmunoassay using previously described techniques (38). Glucagon was measured by radioimmunoassay with a double-antibody procedure using a kit by Linco (Linco Research, St. Charles, MO). Proinsulin was measured by an enzyme-linked immunosorbent assay specific for proinsulin and proinsulin split products (39). FFAs were measured by a colorimetric method (kit supplied by Wako Industrial, Osaka, Japan).

For the determination of 3-[<sup>3</sup>H]glucose SA, plasma was deproteinized with Ba(OH)<sub>2</sub> and ZnSO<sub>4</sub>. An aliquot of the supernatant was then evaporated to dryness to eliminate tritiated water. After addition of water and liquid scintillation solution, the radioactivity from 3-[<sup>3</sup>H]glucose was counted by liquid scintillation spectrometry. An external standard was used for quench corrections. Aliquots of the

infused 3-[<sup>3</sup>H]glucose and of the labeled glucose infusate were assayed together with the plasma samples.

**Statistical methods.** The data were expressed as mean  $\pm$  SE. Two-way analysis of variance for repeated measurements followed by Tukey's  $t$  test was performed for differences between experimental groups during the basal period and during the 150- to 180-min hyperinsulinemic period. A  $P$  value  $<0.05$  was regarded as significant. Calculations were performed with SAS software (SAS Institute, Cary, NC).

**RESULTS**

All mean values reported below for the baseline period are the means of times -40 to 0 min, and for the hyperinsulinemic period, the means of times 150–180 min (i.e., the final 30 min of the clamp).

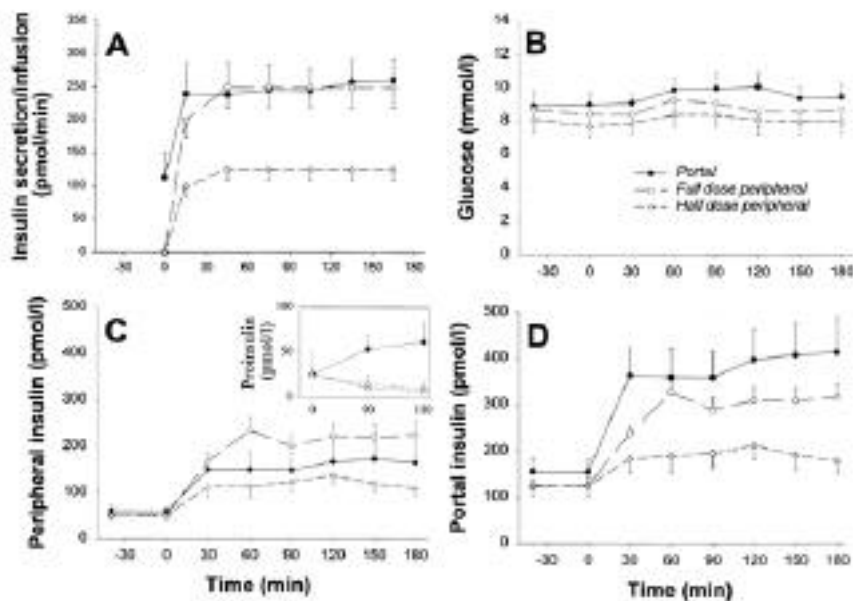
**Insulin infusion/secretion rates, plasma glucose, peripheral insulin, proinsulin, and calculated portal insulin.** In POR, the calculated insulin secretion rate rapidly reached a plateau ( $252.6 \pm 21.6$  pmol/min) by ~30 min after beginning the tolbutamide infusion (Fig. 1A). Its time course was mimicked by PER and 1/2 PER.

Fasting (basal) plasma glucose levels (Fig. 1B) were higher ( $P < 0.05$ ) in POR and PER ( $9.2 \pm 0.3$  and  $8.7 \pm 0.3$  mmol/l) than in 1/2 PER ( $8.0 \pm 0.3$  mmol/l) (NS for POR vs. PER). Glucose levels were clamped at isoglycemia throughout the studies. During the final 30 min of the clamp, the glucose was higher in POR ( $9.4 \pm 0.4$  mmol/l) than in PER and 1/2 PER ( $8.7 \pm 0.3$ ,  $P < 0.05$ , and  $8.0 \pm 0.3$  mmol/l,  $P < 0.001$ ) (NS for PER vs. 1/2 PER).

C-peptide levels (not shown) rose from  $636 \pm 37$  to  $1164 \pm 60$  pmol/l in POR and decreased slightly from  $654 \pm 41$  to  $589 \pm 66$  pmol/l in PER and from  $609 \pm 44$  to  $501 \pm 61$  pmol/l in 1/2 PER.

The peripheral insulin level (Fig. 1C) increased to a greater extent ( $P < 0.05$ ) in PER (from basal level of  $49.3 \pm 3.6$  to  $208.3 \pm 19.0$  pmol/l in the final 30 min of clamp) than in POR (from  $60.5 \pm 6.2$  to  $157.9 \pm 29.1$  pmol/l) or 1/2 PER ( $49.3 \pm 5.0$  to  $107.7 \pm 11.2$  pmol/l,  $P < 0.001$ ). The insulin levels in the final 30 min in POR were higher than those in 1/2 PER ( $P < 0.05$ ).

Proinsulin levels (Fig. 1C inset) increased from basal levels of  $26.4 \pm 8.2$  to  $62.6 \pm 20.7$  pmol/l in POR because of the stimulatory effect of tolbutamide on endogenous insulin secretion, and decreased to  $10 \pm 6$  pmol/l in PER and  $10 \pm 5$  pmol/l in 1/2 PER (because of the suppressive effect of exogenous insulin on endogenous insulin secretion).



**FIG. 1.** Insulin secretion/infusion rates (A), plasma glucose concentrations (B), peripheral insulin concentrations (C) and proinsulin (inset), and estimated portal insulin concentrations (D) versus time for the portal study (●), full-dose peripheral insulin study (○), and half-dose peripheral insulin study (△). Time -40 to 0 min is the basal equilibration period, and 0 to 180 min, the hyperinsulinemic period. Statistical differences between studies are discussed in RESULTS.

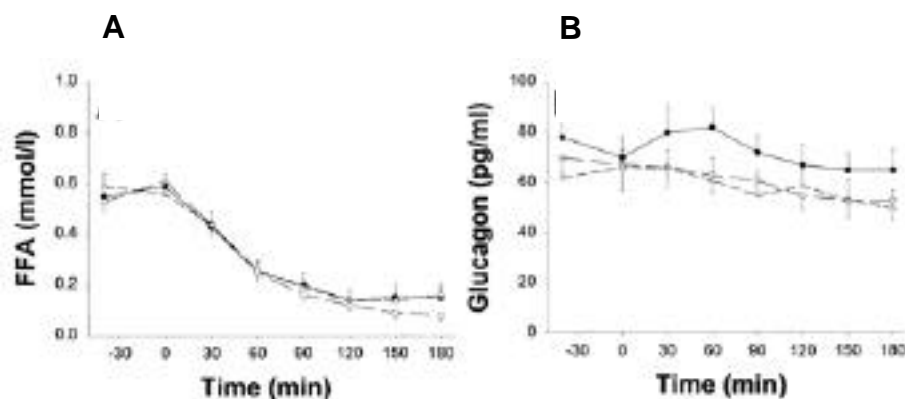


FIG. 2. Plasma FFAs (A) and plasma glucagon concentrations (B) versus time for the portal study (●), full-dose peripheral insulin study (○), and half-dose peripheral insulin study (△). Time -40 to 0 min is the basal equilibration period, and 0-180 min, the hyperinsulinemic period. Statistical differences between studies are discussed in RESULTS.

Calculated portal insulin levels (Fig. 1D) were higher ( $P < 0.001$ ) in the basal state in POR ( $160.1 \pm 28.6$  pmol/l) than in PER and 1/2 PER ( $126.3 \pm 13.8$  and  $127.6 \pm 22.5$  pmol/l) (NS for PER vs. 1/2 PER). The same applied to calculated hepatic insulin levels, which were higher ( $P < 0.001$ ) in the basal state in POR ( $133.5 \pm 23.8$  pmol/l) than in PER and 1/2 PER ( $105.6 \pm 11.7$  and  $106.6 \pm 18.7$  pmol/l) (NS for PER vs. 1/2 PER). During the final 30 min of the clamp, calculated portal insulin levels were higher in POR ( $403.1 \pm 66.5$  pmol/l) than in PER and 1/2 PER ( $312.9 \pm 26.5$ ,  $P < 0.05$ , and  $185.9 \pm 29.2$  pmol/l,  $P < 0.001$ ) ( $P < 0.001$  for PER vs. 1/2 PER). Calculated hepatic insulin levels tended to be higher during the final 30 min of the clamp in POR ( $336.9 \pm 58.5$  pmol/l) than in PER ( $287.7 \pm 26.4$  pmol/l, NS) and were higher than in 1/2 PER ( $166.0 \pm 25.4$  pmol/l,  $P < 0.001$ ) ( $P < 0.001$  for PER vs. 1/2 PER).

**Glucagon and FFAs.** FFA levels (Fig. 2A) were suppressed ( $P < 0.001$ ) during hyperinsulinemia from basal values of  $0.57 \pm 0.03$  mmol/l in POR,  $0.60 \pm 0.03$  mmol/l in PER, and  $0.59 \pm 0.03$  mmol/l in 1/2 PER. The decline in FFAs was slightly less ( $P < 0.05$ ) with POR and 1/2 PER ( $73.7 \pm 3.9$  and  $76.7 \pm 3.0\%$ ) than with PER ( $85.2 \pm 1.9\%$ ) (NS for POR vs. 1/2 PER).

Peripheral glucagon levels (Fig. 2B) decreased to a lesser extent ( $P < 0.001$ ) in POR (by  $15.9 \pm 4.6\%$ , from basal level of  $77.7 \pm 4.4$  to  $65.5 \pm 4.9$  ng/l) compared with PER (by  $22.5 \pm 6.6\%$ , basal  $69.3 \pm 4.2$  to  $52.6 \pm 4.9$  ng/l) and 1/2 PER (by  $24.7 \pm 4.2\%$ , basal  $67.8 \pm 4.5$  to  $51.4 \pm 5.2$  ng/l).

**Glucose infusion rate, 3-[<sup>3</sup>H]glucose SA,  $R_d$ , and endogenous glucose production.** The dextrose infusion rate (Ginf) necessary to maintain euglycemia (Fig. 3A) increased at a similar rate in POR and PER in the first 90 min of the clamp. While the Ginf continued to increase in a linear fashion throughout the 180-min clamp in PER ( $21.3 \pm 2.5$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in the final 30 min), the Ginf declined and reached a plateau at a lower level in POR ( $9.8 \pm 0.9$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.001$ ) and was similar in the final 30 min of the experiment to 1/2 PER ( $11.1 \pm 1.4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) ( $P < 0.001$  for PER vs. 1/2 PER).

Plasma glucose SA (Fig. 3B) increased to a greater extent ( $P < 0.001$ ) during the glucose clamp in POR ( $53.1 \pm 4.0\%$ ) than in PER and 1/2 PER ( $33.4 \pm 5.1$  and  $39.1 \pm 5.0\%$ ) (NS for PER vs. 1/2 PER).

As expected,  $R_d$  (Fig. 3C) rose proportionally to the peripheral insulin levels.  $R_d$  was higher ( $P < 0.001$ ) in the final 30 min of the clamp in PER ( $25.0 \pm 1.8$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) than in POR and 1/2 PER ( $15.5 \pm 0.7$  and  $17.2 \pm 0.8$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) (NS for POR vs. 1/2 PER).

Endogenous glucose production ( $R_a$ ) (Fig. 3D) in the basal period was slightly higher ( $P < 0.05$ ) in POR ( $11.5 \pm 0.4$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) than in PER and 1/2 PER ( $10.6 \pm 0.3$  and  $10.7 \pm 0.2$   $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) (NS for PER vs. 1/2 PER).  $R_a$  decreased to a lower steady-state level in the final 30 min of the clamp (Table 2) in PER than in POR and 1/2 PER ( $P < 0.05$ ) (NS for POR vs. 1/2 PER). As can be seen in Fig. 3 and Table 2, the early suppression of  $R_a$  (between 60 and 90 min of the clamp) was greater ( $P < 0.001$ ) in POR than in PER and 1/2 PER (NS for PER vs. 1/2 PER).

## DISCUSSION

In the present study of subjects with untreated type 2 diabetes, there was a more rapid initial suppression of glucose production with portal versus peripheral insulin, but quantitatively greater suppression with peripheral insulin delivery in the final 30 min of the 180-min clamp. In contrast with our studies in nondiabetic humans and dogs (4-7), however, glucose production was not more suppressed with portal vs. half-dose peripheral insulin in the final 30 min of the clamp, despite much higher hepatic insulin levels with portal insulin. In these patients, the initial direct suppressive effect of insulin could still be detected, while the steady-state direct effect could not be detected. This is the first study in humans with type 2 diabetes to characterize insulin resistance to the direct suppressive effect of insulin on hepatic glucose production.

Suppression of glucose production has been shown to be due to direct hepatic suppression initially (by 15 min), with the peripheral indirect effect being somewhat delayed (by ~1 h) (7,13,40). The initial direct suppressive effect of insulin in dogs has been shown by others to be due to rapid suppression of hepatic glycogenolysis and not gluconeogenesis (7,13,40). The delayed suppression of GP is thought to be mediated not only by indirect mechanisms, but also by a direct hepatic effect of insulin (5,25). The abnormality found in these patients with type 2 diabetes, in contrast with our earlier studies in nondiabetic individuals (5), was the absence of the direct effect of insulin in suppressing GP after 150 min of the clamp. One may argue that although the absolute GP was identical in the last 30 min of the clamp in the POR and 1/2 PER studies, the reduction from basal (as shown in Table 2) tended to be greater in the POR versus the PER study. This difference, however, was not significant, and was mainly due to small differences in basal GP rates between the studies. It is also noteworthy that GP was not maximally suppressed in either study, and therefore could

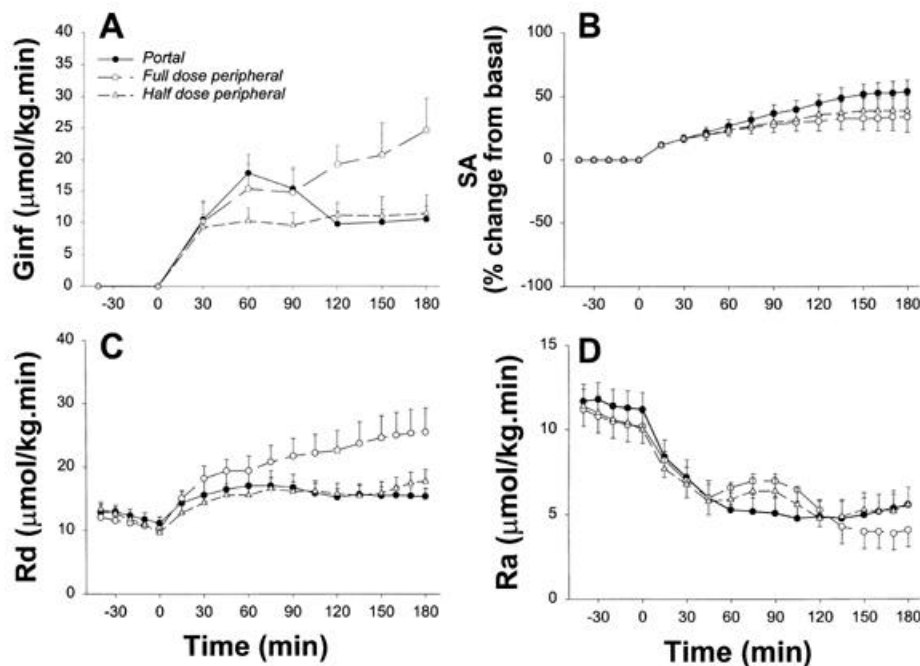


FIG. 3. Ginf (A), 3-[<sup>3</sup>H]glucose SA (B), *R<sub>d</sub>* (C), and endogenous glucose production (*R<sub>a</sub>*) (D) versus time for the portal study (●), full-dose peripheral insulin study (○), and half-dose peripheral insulin study (△). Time -40 to 0 min is the basal equilibration period, and 0–180 min, the hyperinsulinemic period. Statistical differences between studies are discussed in RESULTS.

have been suppressed to a greater extent in the portal insulin study if there had not been resistance to the direct suppressive effect of insulin. The absence of a greater suppressive effect of portal versus half-dose peripheral insulin in the final 30 min of the clamp, despite 2.2-fold higher portal insulin concentrations in the former, suggests that individuals with type 2 diabetes are resistant to the delayed direct suppressive effect of insulin on steady-state GP. Interestingly, this abnormality occurs in type 2 diabetes despite the hyperglucagonemia that has been described in type 2 diabetes (41), which theoretically would be expected to increase the sensitivity of the liver to the direct hepatic effects of insulin on hepatic GP (6,14,15). Our studies in depancreatized dogs also failed to show a direct effect of insulin on GP (26), while those in non-diabetic dogs showed a direct effect (25). We have recently reported that restoration of euglycemia acutely restores the direct hepatic effect of insulin on GP in depancreatized dogs (27), suggesting that hyperglycemia diminishes rather than accentuates the effect of portal insulin.

The patients in the present study for the most part had mild type 2 diabetes and normal basal rates of glucose production. Unfortunately, the sample size is too small to draw conclusions regarding diabetes severity and the effects of portal and peripheral insulin on GP. There were no significant correla-

tions in this respect. It is quite possible that the findings would have been different if we had studied patients with more marked hyperglycemia, although intuitively, based on our findings in depancreatized dogs discussed above (27), we would have expected the defect to be even more severe. In the present study, we did not measure glucose derived from glycogenolysis versus gluconeogenesis and can only speculate, therefore, based on the observations of others who have indicated that the direct hepatic suppressive effect of insulin on GP is due to a reduction of glucose derived from glycogenolysis (7,13,40), that the blunted suppression of steady-state GP in this case was due to insulin resistance at the level of regulation of glycogenolysis.

The finding of quantitatively greater suppression of GP in the final 30 min of the clamp with peripheral versus equidose portal insulin, where the peripheral insulin concentration was 1.3-fold higher but portal insulin was 1.3-fold lower in the peripheral insulin study, confirms earlier findings by our group and those of a number of other investigators that insulin suppresses GP by an indirect mechanism in proportion to its peripheral venous concentration (3,5–7,9–13, 15,25,26,40,42). There is now abundant evidence that insulin's suppressive effect on peripheral tissue lipolysis, with reduction in plasma FFAs, is an important signal medi-

TABLE 2  
Comparison of glucose production rates in the acute (60–90 min) time period and the steady-state (150–180 min) period of the clamp compared with basal

	Basal	60–90 min	150–180 min
GP in portal study (µmol · kg <sup>-1</sup> · min <sup>-1</sup> )	11.6 ± 0.5	5.2 ± 0.6 (-56.5 ± 3.8)	5.3 ± 0.5 (-56.3 ± 3.2)
GP in peripheral study (µmol · kg <sup>-1</sup> · min <sup>-1</sup> )	10.6 ± 0.3*	6.9 ± 0.4 (-33.5 ± 4.4)†	4.0 ± 0.4 (-61.0 ± 4.2)*
GP in half-dose peripheral study (µmol · kg <sup>-1</sup> · min <sup>-1</sup> )	10.8 ± 0.2*	6.3 ± 0.3 (-41.8 ± 2.7)†	5.3 ± 0.4 (-49.8 ± 3.7)‡

Data are means ± SE (% suppression from basal). \**P* < 0.05 vs. portal study; †*P* < 0.001 vs. portal study; and ‡*P* < 0.05 vs. peripheral study.

ating the indirect effect of insulin on hepatic GP. First, a strong relationship was found between insulin's suppression of FFAs and GP (7,9,26). Second, prevention of the insulin-mediated decline in plasma FFAs by intravenous infusion of a triglyceride emulsion has been shown to limit the insulin-mediated decline in GP (10,11,13). In the present study, we did not directly examine the mechanism of insulin's peripheral effect on GP, but FFAs were suppressed to a slightly greater extent with full-dose peripheral insulin than with portal insulin, consistent with the higher peripheral insulin levels in the former. Resistance to insulin's antilipolytic effect has been described in type 2 diabetes (24,43), but in the present study the peripheral insulin levels were in the high physiologic range and resistance to insulin's antilipolytic effect would not be expected to be detected at these insulin doses. Insulin suppression of glucagon secretion has also been considered as a potential mediator of insulin's indirect effect on hepatic GP. Glucagon stimulates GP, and suppression of plasma glucagon levels results in suppression of GP (44). In our previous studies, we found that glucagon enhanced the direct suppressive effect of insulin on GP (6,14), in addition to mediating part of the extrahepatic effect of insulin on GP (14,26). This finding has been confirmed in a recent study by Mittelman et al. (15). In the present study, glucagon was suppressed to a greater extent with peripheral insulin than with portal insulin. In summary, the delayed indirect effect of insulin on endogenous glucose production, which is mediated by a combination of insulin's peripheral tissue antilipolytic effect and insulin-mediated suppression of glucagon secretion, is still detectable in type 2 diabetes.

We cannot readily explain why glucagon was suppressed to a greater extent with half-dose peripheral insulin than with portal, since the systemic levels of insulin were higher with portal insulin and glucagon secretion is suppressed by insulin in proportion to its systemic concentration. In addition, tolbutamide-stimulated pancreatic insulin secretion in the portal study is expected to exert an additional paracrine suppressive effect on  $\alpha$ -cell glucagon secretion, and we would have anticipated lower glucagon levels in the portal study. Most of the differences between portal and half-dose peripheral studies can be accounted for by differences in the basal glucagon levels, with higher basal levels occurring in the portal study. We cannot readily account for this difference in basal glucagon levels between the studies but believe that this difference in glucagon levels is not responsible for the major finding of the study, the lack of difference in suppression of steady-state glucose production between portal and half-dose peripheral insulin. In fact, insofar as glucagon has been shown to potentiate the direct hepatic suppressive effect of insulin (6,14), it is all the more remarkable that there was no greater suppression of GP with portal versus half-dose peripheral insulin in the face of higher glucagon concentrations.

The peripheral insulin level in the portal study was higher than anticipated and was not well matched with that in the half-dose peripheral study. One possible explanation was the higher proinsulin level, since there is 41% cross-reactivity with proinsulin in the insulin assay used in this study. Therefore the peripheral insulin levels reported during the tolbutamide infusion overestimate true insulin concentrations by  $\sim 26$  pmol/l (peripheral proinsulin concentration =  $62.6$  pmol/l  $\times 41\%$ ). This difference does not totally account for the difference in peripheral insulin concentrations between the portal and half-

dose peripheral insulin studies, which was  $\sim 54$  pmol/l. Less than 50% first-pass hepatic insulin clearance in the portal study in these subjects with diabetes could have accounted for the remainder of the difference in peripheral insulin concentrations. During the full- and half-dose peripheral insulin infusion studies, endogenous insulin secretion was suppressed, and consequently proinsulin levels were extremely low (at  $\sim 10$  pmol/l) during the peripheral insulin studies. However, assuming minimal first-pass proinsulin removal by the liver, a maximum portal-peripheral proinsulin gradient of 5, and an  $\sim 10\%$  biological activity of proinsulin (45), the effect of proinsulin in the portal study is no more than the equivalent of  $31.5$  pmol/l portal insulin ( $63$  pmol/l peripheral proinsulin  $\times 5 \times 10\%$ ). Nevertheless, the higher than anticipated peripheral insulin levels in the portal study only strengthen the finding that there is a diminished direct effect of insulin on GP in the final 30 min of the clamp, since portal insulin levels were even higher than expected (2.2-fold higher than in the half-dose peripheral insulin study), with no greater suppression of GP compared with the half-dose peripheral insulin study. The higher peripheral insulin and proinsulin levels in the portal study, however, may have masked potentially greater differences in GP between the equidose peripheral and portal studies.

The increase in proinsulin with tolbutamide is not expected to significantly affect our calculations of insulin secretion rates from peripheral C-peptide levels. First, the molar concentration of proinsulin to C-peptide was 1:18 in this study. Second, there is  $<15\%$  cross-reactivity with proinsulin in this C-peptide assay.

In the present study, we have used HPLC-purified 3- $^3$ H]glucose to avoid artifacts due to nonglucose contaminants in the measurement of hepatic GP (28). We attempted to minimize the change in SA during the glucose clamp by adopting the hot Ginf method (31,32). However, plasma glucose SA increased in all studies because of overestimation of tracer infusion rates, more so in the portal than in the peripheral insulin studies. Since the rise in SA was slow and reached a plateau before the final 30 min of the clamp, it is unlikely to have artifactually affected our results and major conclusions (46).

In conclusion, we have shown that patients with type 2 diabetes are resistant to the direct hepatic suppressive effect of insulin, while the indirect peripherally mediated suppression of glucose production appears to be grossly intact. What are the implications for insulin treatment of individuals with type 2 diabetes? One may be tempted to speculate that, in the face of hepatic insulin resistance, the portal delivery of insulin may be associated with no additional therapeutic benefit when compared with peripheral insulin delivery. We caution, however, that our study specifically investigated the acute effects of insulin and should not be extrapolated to the chronically insulin-treated patient with type 2 diabetes. Peripheral hyperinsulinemia is still found in humans treated chronically with subcutaneous insulin, indicating that with systemic insulin administration adequate glycemic control cannot be achieved without concomitant peripheral hyperinsulinemia. Further studies will be needed to determine whether this hepatic resistance to acute insulin administration is secondary to the hyperglycemia per se and can be reversed if the patients are rendered euglycemic for a few weeks prior to the study. We have speculated that the insulin resistance is at the level of glycogenolysis, but this too needs to be directly investigated in future studies.

## ACKNOWLEDGMENTS

We would like to acknowledge the funding provided by the Canadian Diabetes Association (G.F.L., supported by a grant in memory of the late George and Vesta Davidge), Juvenile Diabetes Foundation (A.G., 193135), and Medical Research Council of Canada (M.V. and A.G., MT2917). A.C. is the recipient of a Juvenile Diabetes Foundation International fellowship. G.F.L. is a Career Investigator of the Heart and Stroke Foundation of Canada.

The expert technical assistance of Debbie Bilinski, Loretta Lam, and Linda Szeto is acknowledged with appreciation.

## REFERENCES

- Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH: Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55:1278-1283, 1975
- Eaton RP, Allen RC, Schade DS: Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. *J Clin Endocrinol Metab* 56:1294-1300, 1983
- Ader M, Bergman RN: Peripheral effects of insulin dominate suppression of fasting hepatic glucose production. *Am J Physiol* 258:E1020-E1032, 1990
- Lewis GF, Giacca A, Vranic M, Steiner G: Direct and indirect effects of insulin on hepatic glucose and very low density lipoprotein (VLDL) production. *Curr Opin Endocrinol Diab* 5:235-245, 1998
- Lewis GF, Zinman B, Groenewoud Y, Vranic M, Giacca A: Hepatic glucose production is regulated both by direct hepatic and extrahepatic effects of insulin in humans. *Diabetes* 45:454-462, 1996
- Lewis GF, Vranic M, Giacca A: Glucagon enhances the direct suppressive effect of insulin on hepatic glucose production in humans. *Am J Physiol* 272:E371-E378, 1997
- Sindelar DK, Balcom JH, Chu CA, Neal DW, Cherrington AD: 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
- Bergman RN: New concepts in extracellular signaling for insulin action: the single gateway hypothesis. *Rec Progr Horm Res* 52:359-387, 1997
- Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44:1038-1045, 1995
- Rebrin K, Steil GM, Mittelman SD, Bergman RN: Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. *J Clin Invest* 98:741-749, 1996
- Lewis GF, Vranic M, Harley P, Giacca A: Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. *Diabetes* 46:1111-1119, 1997
- Lewis GF, Vranic M, Giacca A: Role of free fatty acids and glucagon in the extrahepatic effect of insulin on glucose production in humans. *Am J Physiol* 275:E177-E186, 1998
- Sindelar DK, Chu C, Rohlie M, Neal DW, Swift LL, Cherrington AD: 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
- Giacca A, Fisher SJ, McCall RH, Shi ZQ, Vranic M: Direct and indirect effects of insulin in suppressing glucose production in depancreatized dogs: role of glucagon. *Endocrinology* 138:999-1007, 1997
- Mittelman S, Fu YY, Rebrin K, Steil G, Bergman RN: Indirect effect of insulin to suppress endogenous glucose production is dominant, even with hyperglucagonemia. *J Clin Invest* 100:3121-3130, 1997
- Shishko PI, Kovalev PA, Goncharov VG, Zajamy IU: Comparison of peripheral and portal (via the umbilical vein) routes of insulin infusion in IDDM patients. *Diabetes* 41:1042-1049, 1992
- Garvey WT, Olefsky JM, Marshall S: Insulin receptor down-regulation is linked to an insulin-induced postreceptor defect in the glucose transport system in rat adipocytes. *J Clin Invest* 76:22-30, 1985
- Marshall S, Olefsky JM: Effects of insulin incubation on insulin binding, glucose transport, and insulin degradation by isolated rat adipocytes. Evidence for hormone-induced desensitization at the receptor and postreceptor level. *J Clin Invest* 66:763-772, 1980
- Wardzala LJ, Hirshman M, Pofcher E, Horton ED, Mead PM, Cushman SW, Horton ES: Regulation of glucose utilization in adipose cells and muscle after long-term experimental hyperinsulinemia in rats. *J Clin Invest* 76:460-469, 1985
- Radziuk J, Barron P, Najm H, Davies J: The effect of systemic venous drainage of the pancreas on insulin sensitivity in dogs. *J Clin Invest* 92:1713-1721, 1993
- Rizza RA, Mandarino LJ, Genest J, Baker BA, Gerich JE: Production of insulin resistance by hyperinsulinemia in man. *Diabetologia* 28:70-75, 1985
- Marangou AG, Weber KM, Boston RC, Aitken PM, Heggie JC, Kirsner RL, Best JD, Alford FP: Metabolic consequences of prolonged hyperinsulinemia in humans. Evidence for induction of insulin insensitivity. *Diabetes* 35:1383-1389, 1986
- Consoli A: Role of liver in pathophysiology of NIDDM (Review). *Diabetes Care* 15:430-441, 1992
- Groop LC, Bonadonna RC, DelPrato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA: Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. *J Clin Invest* 84:205-213, 1989
- McCall RH, Wiesenthal SR, Shi ZQ, Polonsky K, Giacca A: Insulin acutely suppresses glucose production by both peripheral and hepatic effects in normal dogs. *Am J Physiol* 37:E346-E356, 1998
- Giacca A, Fisher SJ, Shi ZQ, Gupta R, Lickley HL, Vranic M: Importance of peripheral insulin levels for insulin-induced suppression of glucose production in depancreatized dogs. *J Clin Invest* 90:1769-1777, 1992
- Gupta R, Sandhu H, Goh T, Wiesenthal S, Tchepashvili V, Lam L, Giacca A: Euglycemia acutely restores the direct hepatic effect of insulin on glucose production in depancreatized dogs (Abstract). *Diabetes* 44 (Suppl. 1):A41, 1998
- Schwenk WF, Butler PC, Haymond MW, Rizza RA: Underestimation of glucose turnover corrected with high-performance liquid chromatography purification of [ $^3$ H]glucose. *Am J Physiol* 258:E228-E233, 1990
- Lewis GF, Steiner G, Polonsky KS, Weller B, Zinman B: A new method for comparing portal and peripheral venous insulin delivery in humans: tolbutamide versus insulin infusion. *J Clin Endocrinol Metab* 79:66-70, 1994
- Garner CW: Boronic acid inhibitors of porcine pancreatic lipase. *J Biol Chem* 255:5064-5068, 1980
- Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914-924, 1987
- Finegood DT, Bergman RN, Vranic M: Modeling error and apparent isotope discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. *Diabetes* 37:1025-1034, 1988
- Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368-377, 1992
- Finegood DT, Bergman RN: Optimal segments: a method for smoothing tracer data to calculate metabolic fluxes. *Am J Physiol* 244:E472-E479, 1983
- 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
- De Feo P, Perriello G, De Cosmo S, Ventura MM, Campbell PJ, Brunetti P, Gerich JE, Bolli GB: Comparison of glucose counterregulation during short-term and prolonged hypoglycemia in normal humans. *Diabetes* 35:563-569, 1986
- Campora JL, Reynolds TB: The hepatic circulation. In *The Liver. Biology and Pathobiology*. Arias IM, Popper H, Schachter D, Shafritz DA, Eds. New York, Raven Press, 1982, p. 627-645
- Faber OK, Binder C, Markussen J, Heding LG, Naithani VK, Kuzuya H, Blix P, Horwitz DL, Rubenstein AH: Characterization of seven C-peptide antisera. *Diabetes* 27 (Suppl. 1):170-177, 1978
- Hartling SG, Dinesen B, Kappelgaard AM, Faber OK, Binder C: ELISA for human proinsulin. *Clin Chim Acta* 156:289-297, 1986
- Sindelar DK, Chu CA, Neal DW, Cherrington AD: Interaction of equal increments in arterial and portal vein insulin on hepatic glucose production in the dog. *Am J Physiol* 273:E972-E980, 1997
- Baron AD, Schaeffer L, Shragg P, Kolterman OG: Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetes. *Diabetes* 36:274-283, 1987
- Prager R, Wallace P, Olefsky JM: Direct and indirect effects of insulin to inhibit hepatic glucose output in obese subjects. *Diabetes* 36:607-611, 1987
- Groop LC, Saloranta C, Shank M, Bonadonna RC, Ferrannini E, DeFronzo RA: The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 72:96-107, 1991
- Shi ZQ, Wasserman D, Vranic M: Metabolic implications of exercise and physical fitness in physiology and diabetes. In *Ellenberg and Rifkin Diabetes Mellitus*. Porte D, Sherwin R, Eds. Norwalk, CT, Appleton and Lange, 1997, p. 653-687
- Galloway JA, Hooper SA, Spradlin CT, Howey DC, Frank BH, Bowsher RR, Anderson JH: Biosynthetic human proinsulin. Review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies, and clinical trial experience (Review). *Diabetes Care* 15:666-692, 1992
- Fisher SJ, Shi ZQ, Lickley HLA, Efendic S, Vranic M, Giacca A: A moderate decline in specific activity does not lead to an underestimation of hepatic glucose production during a glucose clamp. *Metabolism* 45:587-593, 1996.