Determination of Portal Insulin Absorption From Peritoneum via Novel Nonisotopic Method

JEAN-LOUIS SELAM, RICHARD N. BERGMAN, DENIS RACCAH, NATHALIE JEAN-DIDIER, JAMES LOZANO, AND M. ARTHUR CHARLES

The absorption mechanism of insulin administered in the peritoneal cavity (IP) is of current interest because of the near availability of implantable insulin-infusion devices for the treatment of diabetes. To determine the fraction of insulin absorbed by the portal circulation after IP administration, a novel nonisotopic method is described. Conscious fasting diabetic dogs were studied at normoglycemia via the euglycemic insulin-clamp method. Posthepatic appearance of insulin and C-peptide were measured in peripheral blood during IP or intravenous (IV) equimolar infusion of insulin and C-peptide at two sequential 3-h infusion rates of 3.2 and 12.8 pmol · kg^{-1} · min^{-1}. Prior studies have shown that 40–60% of portal insulin is extracted at first pass by the liver, whereas C-peptide is not extracted. Thus, the fraction (*F*) of IP insulin not taken up by liver at first pass and consequently the fraction absorbed by the portal circulation can be derived from insulin (I) and C-peptide (C) plasma concentration values at steady state with a monocompartmental model where $F = (I_{IP}/I_{IV})(C_{IV}/C_{IP})$. The mean ± SE value of *F* was 49.7 ± 8.8%. Glucose disappearance rates were lower with IP than IV infusion but similar when peripheral insulin levels were matched. We conclude that IP insulin is almost entirely absorbed by the portal circulation and induces lower glucose disappearance rates than IV insulin because of lower peripheral circulating insulin levels. Whether these properties make the IP route a more appropriate route for insulin therapy than the subcutaneous or IV routes remains to be established. ***Diabetes*** **39:1361–65, 1990**

The determination of a significant portal drainage of insulin administered in the peritoneal cavity (IP) indicates that the peritoneum is a more physiological route for insulin administration than the subcutaneous or intravenous (IV) routes. Data to support this concept would provide a more scientific basis for the use of implantable delivery devices with chronic IP insulin delivery. Some of these devices have proved to be reasonably safe and feasible in humans with the IV and the IP routes and are in final stages of design for clinical use (1–4). A few animal studies with invasive isotopic techniques have shown higher portal than systemic insulin levels after IP insulin administration (5,6). Others have reported the portal absorption of IP insulin to be insignificant compared with the contribution of the peripheral, e.g., lymphatic, system (7). The contribution of portal absorption of IP insulin has been found to vary from 25 to 61%, depending on the amount of insulin administered (8). Thus, the question of the relative absorption of IP insulin via the portal versus the systemic circulation remains unclear.

In this study, we determined the fraction of insulin absorbed by the portal circulation after IP insulin administration with a novel nonisotopic method in diabetic dogs (9). Insulin and C-peptide were measured in peripheral blood during equimolar IP infusion of insulin and C-peptide and during an identical systemic infusion. Because insulin entering the portal circulation would be 40–60% degraded during its first pass through the liver, the extent to which plasma insulin is less during IP than IV infusion would be indicative of portal absorption of the peptide (10–12). Assuming complete absorption from the peritoneal acvity, C-peptide should be identical with both routes because C-peptide is not degraded during its liver pass (13–16). Quantitative hepatic extraction and portal absorption of IP insulin were calculated from the plasma values of C-peptide and insulin at steady state via monocompartmental analysis.

RESEARCH DESIGN AND METHODS

The study was approved by the Animal Research Committee at the University of California, Irvine. Five adult beagles

From the Diabetes Research Program and the Department of Medicine, University of California, Irvine; and the Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, California.

Address correspondence and reprint requests to Dr. Jean-Louis Selam, Department of Diabetology, Hotel-Dieu, 1 Place du Parvis Notre-Dame, 75181 Paris Cedex 4, France.

Received for publication 28 November 1989 and accepted in revised form 15 May 1990.

weighing 13 ± 1 kg (mean ± SE) were studied at least 1 mo after insulin-dependent diabetes was induced with 60 mg/kg IV alloxan. Dogs were treated with a combination of human short-acting and long-acting insulin given subcutaneously once daily. The day before the experiment, only short-acting insulin was given subcutaneously every 6 h.

Experimental design. Each dog underwent two randomly ordered procedures at 2- to 4-wk intervals (Fig. 1). These intervals were chosen to allow blood volume recovery. In one of the procedures, human insulin and human C-peptide were infused concomitantly at equimolar rates into the peritoneal cavity via a percutaneous temporary catheter. In the other procedure, an identical infusion was performed IV in the saphenous vein.

The following equation was used to determine the fraction (F) of IP insulin not extracted by the liver

$$F = (I_{IP}/I_{IV})(C_{IV}/C_{IP})$$

where I and C are insulin and C-peptide plasma concentration values, respectively, at steady state. The equation is based on the fact that, in the dog, insulin is extracted by the liver at first pass, but C-peptide is not (13–15). The eventual residual dog insulin and C-peptide secretion would affect both components of the ratios involved in F and therefore not alter the overall results. The contribution of the portal circulation to IP insulin absorption is then derived from F, knowing that 40–60% of portal insulin is extracted by the liver at first pass (10–12). The mathematical model adapted from Vølund et al. (10) is shown in Fig. 1 and developed in the Appendix.

Experimental procedure. One day before each IP experiment, a temporary IP catheter was inserted percutaneously through the midabdomen by use of a peel-away cannula (17). The procedure was performed under short general barbiturate anesthesia. Food and subcutaneous insulin administration were discontinued at least 12 h before the experiment. On the day of the experiment, dogs were conscious, comfortably immobilized in a Pavlov frame, and not fed. An IV catheter was inserted in the jugular vein for blood sampling. A second IV catheter was inserted in the saphenous vein for 50% dextrose infusion with a Harvard 22 pump. Another Harvard pump was filled with a freshly prepared solution containing 6410 pmol/ml each of insulin and C-peptide and was connected either to a third IV catheter inserted in the saphenous vein in the IV experiment or to the IP catheter in IP experiments.

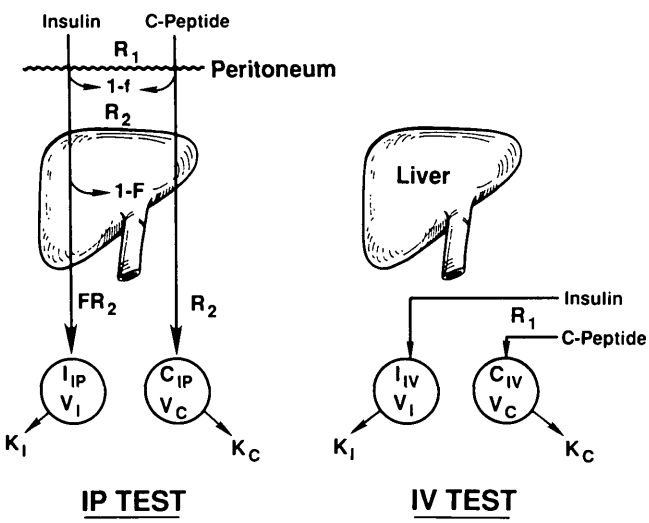

FIG. 1. Representation of mathematical model for calculating fraction (F). R, infusion rate; I, insulin; C, C-peptide; V, volume; IP, intraperitoneal; IV, intravenous; K, 1st-order elimination constants.

Preparation of insulin was based on an insulin molecular weight of 5734 and an insulin weight of the solution used (human U-400, Hoechst PH21, Frankfurt, FRG) of 14.7 mg/ml. Thus, 6410 pmol = 1 U insulin. Preparation of C-peptide was based on a C-peptide molecular weight of 3617, a concentration of the product used (human C-peptide, Bachem, Torrance, CA) of 83%, and a purity of >99%. During a 2-h prestudy period, blood glucose levels were measured every 10 min. In a few cases, small doses of insulin (0.1–0.5 U IV) were injected during the prestudy period to lower the blood glucose concentrations in the 4.4- to 5.5-mM range.

The study began only after blood glucose values were maintained in the above range for at least 30 min after the last IV insulin injection. At time 0, two sequential insulin–C-peptide infusion periods of 3 h each were performed. Each constant-infusion sequence was preceded by a priming dose calculated according to a formula proposed by Rizza et al. (18)

$$\text{insulin dose (mU)} = \text{weight (kg)} \times \text{desired change of plasma insulin (mU/L)}/10$$

A basal initial plasma insulin level of 10 mU/L and a distribution volume of 10% body weight was assumed. For the first infusion sequence, the constant infusion rate was 3.2 pmol · kg^{-1} · min^{-1}, i.e., providing an insulin rate of 0.5 mU · kg^{-1} · min^{-1}, and was preceded by a bolus equivalent to 5 mU/kg. The second infusion rate was set at 12.8 pmol · kg^{-1} · min^{-1}, i.e., corresponding to an insulin rate of 2 mU · kg^{-1} · min^{-1}, and was preceded by a bolus equivalent to 8 mU/kg. Every 30 min and then every 10 min during the last 30 min of each infusion sequence, venous blood samples were taken and transferred to heparinized tubes for plasma free-insulin determinations and to Trasylol-EDTA heparinized tubes for C-peptide determinations. Blood glucose was measured every 10 min, and IV dextrose infusion was adjusted to maintain blood glucose values in the 4.4- to 5.5-mM range.

Methods of analysis. Blood glucose levels were measured on whole–venous blood samples by a reflectance meter (Glucometer M, Ames). Human plasma free-insulin values were determined by radioimmunoassay (Incstar) after immediate antibody extraction by polyethylene glycol, as routinely performed in our laboratory (19,20). The limits of detection are 1 mU/L; interassay and intra-assay variations were <5 and <8%, respectively. C-peptide levels were measured in our laboratory by radioimmunoassay (21). The limits of detection are 0.02 pmol/ml; interassay and intra-assay variations were <9 and <5%, respectively. This assay does not detect dog C-peptide.

Data are expressed as means ± SE. Comparisons were assessed via paired t tests.

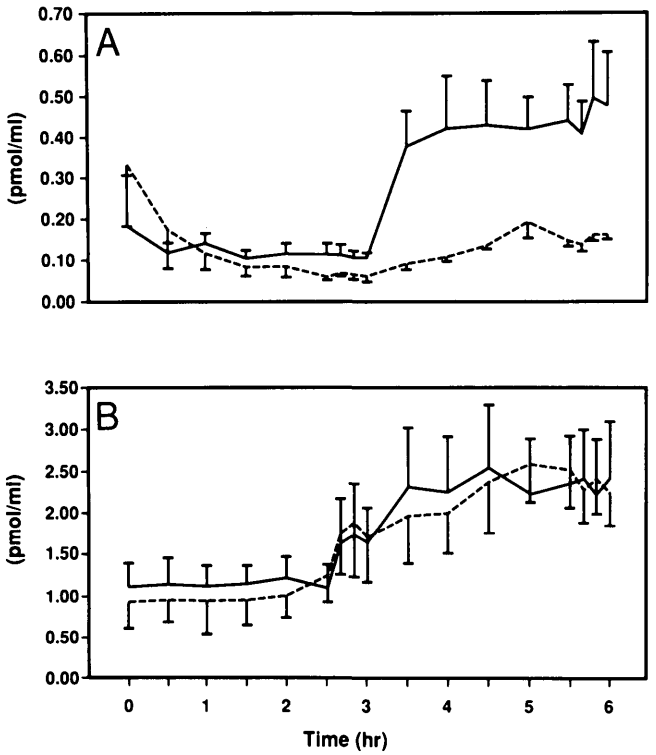

FIG. 2. Circulating insulin (*A*) and C-peptide (*B*) levels during intraperitoneal (*dashed lines*) and intravenous (*solid lines*) insulin–C-peptide infusion experiments. Values are means + SE.

RESULTS

Insulin–C-peptide infusion data. Plasma C-peptide and free-insulin levels during the IV and IP experiments are shown in Fig. 2. Values reached a plateau 30 min after IV infusion but 2 h after IP infusion. C-peptide values during the periods of *F* determination, i.e., the last 30 min of each 3-h sequence, were similar with IV and IP infusion. Circulating free-insulin levels were lower during IP infusion: 0.06 ± 0.01 pmol/ml (IP) vs. 0.10 ± 0.01 pmol/ml (IV) during the 3.2 pmol · kg^{-1} · min^{-1} infusion sequences (NS) and 0.14 ± 0.01 pmol/ml (IP) vs. 0.43 ± 0.02 pmol/ml (IV) during the 12.8 pmol · kg^{-1} · min^{-1} infusion sequences ($P < 0.05$). *F* calculations are shown in Table 1. Overall *F* was 49.7 ± 8.8%, and *F* values were similar at the low and high insulin–C-peptide infusion rates. Therefore, the fraction of insulin extracted by the liver at first pass is 50.3%, indicating a complete intraportal absorption of IP insulin.

Glucose-clamp data. Clamped blood glucose levels were 5.0 ± 0.5 and 5.1 ± 0.6 mM during the IV and IP studies, respectively (NS; Fig. 3). Glucose infusion rates during the last 30 min of the 3.2-pmol · kg^{-1} · min^{-1} infusion sequence were nil during the IP and 1.97 ± 0.82 mg · kg^{-1} · min^{-1} during the IV insulin–C-peptide infusion ($P < 0.001$). Glucose infusion rates were 3.80 ± 0.89 and 16.53 ± 3.64 mg · kg^{-1} · min^{-1} with the 12.3 pmol · kg^{-1} · min^{-1} IP and IV infusion rates, respectively ($P < 0.02$).

Glucose infusion rates were not significantly different during the 3.2 pmol · kg^{-1} · min^{-1} IV and the 12.3 pmol · kg^{-1} · min^{-1} IP infusion sequence, i.e., when peripheral circulating free-insulin levels were similar (Table 1). Glucose infusion rate was 1.97 mg · kg^{-1} · min^{-1} for a free-insulin level of 0.10 pmol/ml during IV infusion and 3.80 mg · kg^{-1} · min^{-1} for a free-insulin level of 0.14 pmol/ml during IP infusion.

DISCUSSION

This study confirms that IP insulin is absorbed mainly by the portal circulation (5,6,8). Our results extend the data by documenting the portal absorption of IP insulin in a more physiological setting: circulating insulin levels during the low- and high-infusion sequences were in the range of values currently observed in diabetic patients in the fasting and postprandial states, respectively. Furthermore, our method does not involve portal or posthepatic catheterization or radioactive isotope administration. The only invasive procedure, i.e., the placement of an IP catheter, could be avoided if the subject is equipped with an implantable IP insulin-delivery device, e.g., an implantable pump. Therefore, this method could be directly applicable to patients treated with an implantable IP pump.

The method we used is based on the following original concept, formulated by one of us (R.N.B.): 40–60% of portal

TABLE 1
Plasma hormone levels and fraction values

Dog	Infusion rate (pmol · kg^{-1} · min^{-1})	Insulin (pmol/ml) IV	Insulin (pmol/ml) IP	C-peptide (pmol/ml) IV	C-peptide (pmol/ml) IP	*F* (%)
1	3.2	0.09	0.05	0.55	0.76	40
	12.8	0.62	0.15	1.10	1.55	17
2	3.2	0.18	0.04	0.60	0.56	24
	12.8	0.67	0.18	1.24	1.17	28
3	3.2	0.09	0.06	2.76	3.12	59
	12.8	0.45	0.10	4.25	3.50	28
4	3.2	0.06	0.09	1.49	2.33	96
	12.8	0.22	0.15	1.98	2.40	56
5	3.2	0.08	0.04	2.34	1.46	80
	12.8	0.21	0.13	3.33	3.00	69
Mean ± SE	3.2	0.10 ± 0.02	0.06 ± 0.01	1.53 ± 0.46	1.65 ± 0.48	59.8 ± 14.0
						49.7 ± 8.8
	12.8	0.43 ± 0.02	0.14 ± 0.01*	2.38 ± 0.61	2.39 ± 0.40	39.6 ± 10.9

Values are averages of plasma concentration measured at 4 10-min intervals during the infusion. IV, intravenous; IP, intraperitoneal; *F*, fraction of IP insulin not taken by liver at 1st passage.
*$P < 0.05$ vs. IV insulin. All other comparisons not significant.

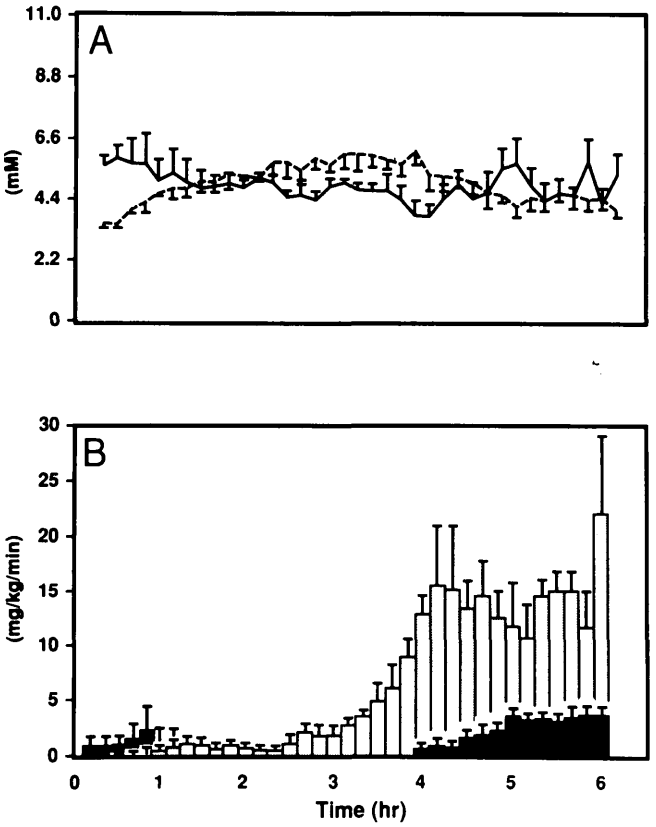

FIG. 3. Blood glucose levels (*A*) and glucose infusion rates (*B*) in intraperitoneal (*dashed line*, *solid bars*) and intravenous (*solid line*, *shaded bars*) experiments. Values are means + SE.

insulin is extracted by the liver at first pass (10–12), but portal C-peptide is not (13–15). Thus, it is possible to evaluate the fraction of IP insulin extracted by the liver at first pass by use of an equimolar IP infusion of insulin and C-peptide and by comparing the posthepatic plasma hormone levels. By use of a monocompartmental model and combining the IP data with those of a similar IV experiment at steady state, differences in the systemic distribution and degradation of C-peptide and insulin can be eliminated. Thus, the plasma levels of insulin and C-peptide reflect only the differences in their liver extraction. The contribution of the portal circulation to the absorption of IP insulin is then easily derived by comparing the extracted insulin fraction after IP administration with that reported after portal insulin. Plasma hormone steady states were attained faster by IV than IP but unexpectedly not slower for C-peptide than for insulin, maybe because of a better efficacy of the C-peptide priming dose. At steady state, during the high-dose infusion sequence, we found similar plasma C-peptide levels but 2–3 times lower plasma insulin values during the IP insulin–C-peptide infusion compared with the IV infusion, clearly indicating a liver trapping and therefore portal absorption of IP insulin. During the low-dose infusion, plasma insulin data were less uniform and therefore did not reach significance. Calculations showed that the first-pass liver trapping was 50%, indicating that virtually all IP insulin was absorbed by the portal circulation. Thus, our data indicate that IP insulin administration may be preferable to other routes of administration, e.g., subcutaneous and IV, for chronic treatment of diabetes, because IP insulin absorption follows the portal circulation, i.e., the physiological pathway.

Clinical implications of IP insulin administration over peripheral routes remain to be established. Although not a primary objective of the study, our data provide information on peripheral circulating levels of insulin and effects on glucose metabolism with IP versus IV insulin administration (22–24). Peripheral insulin concentrations were lower with IP insulin, suggesting that chronic IP insulin administration may generate less hyperinsulinemia and therefore be less atherogenic than IV insulin (25). On the other hand, two- to fourfold lower glucose infusion rates were needed during IP than during IV insulin infusion to maintain normoglycemia. This was expected because peripheral insulin levels were lower with IP than IV insulin infusion and, at the circulating insulin levels obtained during the low- and high-infusion sequences, the peripheral glucose utilization is responsible for ~50 and ~80% of overall glucose disappearance, respectively, compared with hepatic glucose output suppression (26). However, specific measurement of hepatic glucose release and uptake are required to confirm our findings.

At matched peripheral circulating insulin levels, glucose infusion rates were similar with IP and IV insulin administration. The reason fourfold rather than just twofold higher IP insulin rates were needed to generate plasma insulin levels similar to those obtained during IV insulin infusion when only 50% of IP insulin is extracted by the liver is unclear. Nevertheless, these findings suggest a higher hepatic insulin concentration during IP than IV insulin administration, although hepatic glucose output suppression is similar. These data are consistent with previous reports and suggest that portal hepatic insulin flow is not the major determinant in hepatic glucose output suppression. Instead, hepatic glucose output may be regulated mainly by peripheral blood via either insulin levels or gluconeogenic substrates (M. Ader and R.N. Bergman, unpublished observations).

We conclude that *1*) IP insulin is almost entirely absorbed by the portal circulation, *2*) our method for measuring hepatic extraction of insulin may be preferable to other more invasive and/or radioisotopic methods especially in humans, and *3*) IP insulin induces lower glucose disappearance rates than IV insulin via lower peripheral circulating insulin levels.

Finally, these data provide a scientific rationale for new methods of insulin therapy that include chronic IP infusion with implantable delivery devices. However, the clinical relevance of IP insulin administration over less physiological methods, e.g., subcutaneous or IV insulin therapy, remains to be determined.

ACKNOWLEDGMENTS

We are indebted to E. Chan, Diabetes Research Program at the University of California, Irvine, for measuring free insulin and C-peptide; B. Francis and W. Walsleger, Biodevices Laboratories, Orange, CA, for care of the dogs; K. Chang for help with blood processing; K. Waxman, R. Watanabe, and H. Soleman for initial input; P. Lord, MiniMed Technologies, Inc., for providing dogs; and J. Sebag for proofing the manuscript.

This study was presented in part at the 1989 meeting of the European Association for the Study of Diabetes, Lisbon, Portugal.

APPENDIX: IP AND IV TESTS

R_1 = infusion rate of C-peptide and insulin.
f = fraction of insulin and C-peptide having passed the peritoneal membrane.
$R_2 = fR_1$.
F = fraction of insulin and C-peptide that is not taken up by liver during first passage.
V_I, V_C = distribution volumes for insulin and C-peptide.
K_I, K_C = first-order elimination constants for insulin and C-peptide.
I_{IP}, C_{IP} = concentrations of insulin and C-peptide in plasma at steady state during the IP infusion experiment.
I_{IV}, C_{IV} = concentrations of insulin and C-peptide in plasma at steady state during the IV infusion experiment.

IP test. We assumed that the peritoneum retains and/or degrades a similar fraction ($1 - f$) of insulin and C-peptide (10–12), if any, and that the liver takes no C-peptide (13–15). With these assumptions, insulin and C-peptide appear in the posthepatic systemic circulation at rates of FR_2 and R_2, respectively. On entering the inferior vena cava from the hepatic vein, the two peptides are exposed to systemic distribution and degradation that are not the same for insulin and C-peptide in the dog (13–16). Although multicompartmental representation for either peptide could be proposed for the analysis of data, we have used a monocompartmental distribution and metabolism model because it constitutes an acceptable approximation (10).

The net rate of appearance of insulin in the systemic blood is

$$V_I(dI_{IP}/dt) = F \cdot fR_1 - k_I I_{IP} V_I \quad \text{(A1)}$$

and the net rate of appearance of C-peptide is

$$V_C(dC_{IP}/dt) = R_2 - k_C C_{IP} V_C \quad \text{(A2)}$$

IV test. Insulin and C-peptide appear in the systemic circulation directly, i.e., no exposure to the peritoneum or first-pass hepatic extraction. Thus, the net rate of appearance of insulin in the systemic blood is

$$V_I(dI_{IV}/dt) = R_1 - k_I I_{IV} V_I \quad \text{(A3)}$$

and the net rate of appearance of C-peptide is

$$V_C(dC/dt) = R_1 - k_C C_{IV} V_C \quad \text{(A4)}$$

After combination, Eqs. A1 and A3 become $F \cdot f = I_{IP}/I_{IV}$, and Eqs. A2 and A4 become $f = C_{IP}/C_{IV}$. Thus, $F = (I_{IP}/I_{IV})(C_{IV}/C_{IP})$.

REFERENCES

1. Point Study Group: One year trial of remote-controlled implantable insulin infusion system in type 1 diabetic patients. *Lancet* 1:866–69, 1988
2. Selam J-L: Development of implantable insulin pumps: long is the road. *Diabetic Med* 5:724–33, 1988
3. Saudek CD, Selam J-L, Pitt HA, Waxman K, Rubio M, Turner D, Jean-Didier N, Charles MA, Fischell RE: A preliminary trial of the programmable implantable medication system for insulin delivery. *N Engl J Med* 321:574–79, 1989
4. Selam JL, Charles MA: Devices for insulin administration. *Diabetes Care* 13:955–79, 1990
5. Schade DS, Eaton P, Davis T, Akiya F, Phinney E, Kubica R, Vaughn E, Pay PW: The kinetics of peritoneal insulin absorption. *Metabolism* 30: 149–55, 1981
6. Nelson JA, Stephen R, Landou ST, Wilson DE, Tyler FH: Intraperitoneal insulin administration produces a positive portal-systemic blood insulin gradient in unanesthetized unrestrained swine. *Metabolism* 31:969–72, 1982
7. Kernstine KH, Rhode TD, Wigness BD, Arens D, Bordedwich P, Dorman F, Buchwald H: Is intraperitoneal insulin delivery more physiologic than intravenous delivery? *Diabetes Nutr Metab.* In press
8. Radziuk J, Pye S, Davies G, Seigler D, Reeves M: Splanchnic and systemic absorption of intraperitoneal insulin (Abstract). *Diabetes Res Clin Pract* 5 (Suppl. 11):24A, 1988
9. Rubin J, Jones Q, Planch A, Rushton F, Bower J: The importance of the abdominal viscera to peritoneal transport during peritoneal dialysis in the dog. *Am J Med Sci* 292:203–208, 1986
10. Vølund A, Polonsky KS, Bergman R: Calculated pattern of intraportal insulin appearance without independent assessment of C-peptide kinetics. *Diabetes* 36:1195–202, 1987
11. Rubenstein AH, Pottenger LA, Mako ME, Getz GS, Steiner DF: The metabolism of proinsulin and insulin by the liver. *J Clin Invest* 51:912–21, 1972
12. Polonsky K, Jaspan J, Emmanouel D, Holmes K, Moosa AR: Differences in the hepatic and renal extraction of insulin and glucagon in the dog: evidence for saturability of insulin metabolism. *Acta Endocrinol* 102:420–27, 1983
13. Polonsky K, Jaspan J, Pugh W, Cohen D, Schneider M, Schwartz T, Moosa AR, Tager H, Rubenstein AH: Metabolism of C-peptide in the dog: in vivo demonstration of the absence of hepatic extraction. *J Clin Invest* 72: 1114–23, 1983
14. Polonsky KS, Rubenstein AH: C-peptide as a measure of the secretion and hepatic extraction of insulin: pitfalls and limitations. *Diabetes* 33:486–94, 1984
15. Polonsky K, Frank B, Pugh W, Addis A, Karrison T, Meier P, Tager H, Rubenstein A: The limitations to and valid use of C-peptide as a marker of the secretion of insulin. *Diabetes* 35:379–86, 1986
16. Polonsky KS, Pugh W, Jaspan JB, Cohen DM, Harrison T, Tager HS, Rubenstein AH: C-peptide and insulin secretion: relationships between peripheral concentration of C-peptide and insulin and their secretion rates in the dog. *J Clin Invest* 74:1821–29, 1984
17. Selam JL, Slingeneyer A, Hedon B, Mares P, Beraud JJ, Mirouze J: Long-term ambulatory peritoneal insulin infusion of brittle diabetes with portable pumps: comparison with intravenous and subcutaneous routes. *Diabetes Care* 6:105–11, 1983
18. Rizza R, Westland RE, Hall LD, Patton GS, Haymond MW, Clemens AH, Gerich JE, Service JF: Effects of peripheral versus portal venous administration of insulin on post-prandial hyperglycemia and glucose turnover in alloxan-diabetic dogs. *Mayo Clin Proc* 56:434–38, 1981
19. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH: Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes* 26–29, 1977
20. Selam JL, Kashyap M, Alberti KGMM, Hanna M, Lozano J, Turner D, Charles MA: Comparison of intraperitoneal and subcutaneous insulin administration on lipids, apolipoproteins, fuel metabolites and hormones in type 1 diabetes mellitus. *Metabolism* 38:908–12, 1989
21. Heding LG: Radioimmunological determinations of human C-peptide in serum. *Diabetologia* 11:541–48, 1975
22. Stevenson RW, Parsons JA, Alberti KGMM: Effect of intraportal and peripheral insulin on glucose turnover and recycling in diabetic dogs. *Am J Physiol* 244:E190–95, 1983
23. Ishida T, Chap Z, Chou J, Lewis RM, Hartley CJ, Entman ML, Field JB: Effects of portal and peripheral venous insulin infusion on glucose production and utilization in depancreatized conscious dogs. *Diabetes* 33:984–90, 1984
24. Micossi P, Cristallo P, Giacca A, Scavinni M, Dono F, Eaton RP, Pozza A: Hepatic glucose production and metabolic clearance rate following intraperitoneal or subcutaneous insulin treatment (Abstract). *Diabetologia* 29:570A, 1986
25. Stolar MW: Atherosclerosis in diabetes: the role of hyperinsulinemia. *Metabolism* 37:1–9, 1988
26. Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630–39, 1981