Portal and Peripheral Vein Immunoreactive Insulin Concentrations Before and After Glucose Infusion

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SUMMARY

Catheterization of the portal vein via the umbilical vein was performed under local anesthesia in twelve nondiabetic subjects prior to exploratory laporatomy for a variety of conditions. Immunoreactive insulin (IRI) in simultaneously obtained portal and peripheral vein plasma was determined before, during, and after a two-minute glucose infusion (25 gm.). Two phases of insulin secretion were apparent from portal vein IRI concentrations. A rapid secretory phase beginning one minute after start of the infusion and lasting three to four minutes was followed by a slower secretory phase beginning approximately ten minutes after start of the glucose infusion. The absolute amount of "big" insulin (proinsulin-like material) in the portal vein was similar during the first phase and the early part of the second phase of insulin secretion. A significant positive correlation between portal vein and peripheral vein IRI responses to glucose was noted. DIABETES *19*:302-06, May, 1970.

Peripheral vein insulin concentrations have been widely used as an index of pancreatic insulin secretion. Insulin concentrations in peripheral veins may not accurately reflect pancreatic secretion, however, since insulin is secreted into the portal system and must traverse the hepatic bed before reaching the periphery. In order to study the relationship between portal vein and peripheral vein insulin concentrations, a relatively simple method of obtaining blood samples from the portal vein via the collapsed umbilical vein was utilized so that simultaneously obtained portal and peripheral vein immunoreactive insulin (IRI) could be compared before and after glucose administration. In addition to the quantitative aspects, qualitative properties of insulin released into the portal system were studied by determining "big" and "little" insulin. Although the patients studied in this report were not normal they were nondiabetic.

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METHODS

Twelve nondiabetic subjects requiring abdominal exploration and hepatic portography were selected for this study. The patients had a variety of conditions requiring surgery, which are listed in table 1 along with information concerning age, sex, race, family history, and determinants of glucose tolerance. Fasting blood glucose determinations were normal in all and a family history of diabetes was obtained in only two subjects. Oral glucose tolerance tests were performed on seven of the patients a month or more following surgery. The three subjects whose glucose disappearance rates were lowest (k values < 3.0) and the two patients with a family history of diabetes were in the tested group and all oral glucose tolerance tests were normal. Liver function tests were normal in all subjects except patient E.G. who had obstructive jaundice due to choledocholithiasis.

After an overnight fast, the patients were taken to the operating room and prepared in the usual fashion for an abdominal exploration. No general anesthetic was given although some of the patients received 5 mg. Valium or 50 mg. Demerol prior to the procedure. A catheter for withdrawal of blood samples was placed in an antecubital vein prior to the operative procedure and the catheter was kept patent by a saline infusion delivered at a slow rate. Under local anesthesia with 1 per cent xylocaine, the umbilical vein was exposed through an extraperitoneal upper abdominal midline incision and cannulated with a size 9 ureteral catheter. The catheter was then passed into the left branch of the portal vein just past its entrance into the liver. After the catheter had been secured in place, blood samples were easily obtained from the portal vein. The catheter was rinsed with saline prior to obtaining each blood sample.

TABLE 1

Patient	Age	Sex	Race	Diabetes family history	K values*	Glucose test†	Diagnosis
H. H.	64	M	Negro	—	11.2	FBS	Abdominal mass—neg. exploration
H. P.	30	M	Caucasian	—	3.0	FBS	Duodenal ulcer
T. D.	35	M	Negro	—	3.8	Oral GTT	Abdominal tuberculosis
M. J.	55	F	Negro	—	3.0	FBS	Cholelithiasis
O. M.	62	M	Caucasian	—	2.4	Oral GTT	Cholelithiasis
E. H.	50	M	Caucasian	—	2.7	Oral GTT	Adenocarcinoma rectum
W. W.	72	M	Caucasian	—	5.6	FBS	Cholecystitis and choledocholithiasis
E. G.	52	M	Caucasian	2 uncles	4.5	Oral GTT	Cholelithiasis and choledocholithiasis
C. J.	59	M	Negro	—	2.3	Oral GTT	Gastric ulcer
L. D.	49	M	Negro	sister	4.2	Oral GTT	Cholelithiasis
A. P.	64	M	Negro	—	5.7	FBS	Adenocarcinoma stomach
E. J.	44	M	Negro	—	3.0	Oral GTT	Adenocarcinoma rectum

*Calculated by the method of Amatuzio and coworkers[4] from blood glucose values at three and fifteen minutes following a 25-gm. glucose infusion.

†FBS was normal in all subjects and oral glucose tolerance tests performed on seven of the patients were normal by the Wilkerson point system.[9]

After two baseline blood samples one minute apart had been obtained simultaneously from both the portal and antecubital veins, 25 gm. glucose was infused at a constant rate over two minutes in the opposite antecubital vein. Blood samples from the portal and antecubital veins were then obtained simultaneously at 1, 3, 5, 8, 10, and 15 min. after completion of the glucose infusion. In seven of the twelve experimental subjects, portal vein blood samples were also obtained during the infusion (60, 90, and 120 seconds after start of the glucose infusion) and at thirty seconds after completion of the infusion. Blood samples were collected in heparinized tubes. Following completion of the investigation dye was injected into the portal vein for radiographic visualization of the hepatic portal system. Then the patients were given a general anesthetic prior to laparotomy.

Blood glucose and plasma IRI were determined on all samples and "big" and "little" insulin were assessed on portal vein samples obtained at one and ten minutes following glucose infusion in selected patients. Immediately after each experiment, protein-free filtrates were made from 0.2 ml. blood for glucose determinations and the plasma was separated by centrifugation and stored in a freezer until time for other assays.

Blood glucose was determined by a glucose oxidase method.[1] Plasma IRI was determined by a double antibody radioimmunoassay method[2] utilizing insulin-I-125 as tracer. Plasma "big" and "little" insulin were assessed by the method of Roth and coworkers[3] except that the protein and salt peaks in fractions from the Sephadex column were marked with I-131 labeled albumin and NaI-131 and that the buffer used for both the column separation of "big" and "little" insulin and the immunoassay was 0.025 M phosphate buffer pH 7.5 with 1 per cent albumin and 0.1 per cent merthiolate. One ml. plasma with tracer albumin-I-131 and NaI-131 was put on a 1 × 50 cm. Sephadex G-50 column. Fractions of 1 to 1.5 ml. were collected by means of an automatic sample collector set up in a cold room at 4° C. The fractions between the labeled protein and salt peaks were analyzed for IRI, and "big" insulin was arbitrarily defined as IRI in fractions from the protein peak to 30 per cent of the distance between the two peaks.

RESULTS

Peripheral and portal vein IRI values in seven subjects before, during, and after glucose infusion are shown in figure 1. The data on five additional subjects are not included in this figure as portal vein samples were obtained only after completion of the glucose infusion in earlier subjects. The peak portal vein IRI concentration occurs rapidly at completion of the glucose infusion (two minutes after start of injection). When glucose is given in this fashion, peak peripheral vein IRI does not occur until three or five minutes after completion of the infusion in the majority of subjects (ten of twelve patients). Peripheral vein samples were not obtained at two and four minutes so the time interval between peripheral and portal vein IRI peaks can be ascertained no more accurately than some time between two and five minutes. Fasting portal vein insulin (36.9 ± 4.4 μU./ml.) was twice that in peripheral vein (16.0 ± 2.9 μU./ml.) whereas approximately a ten-fold difference in the two venous insulin concentrations was observed shortly after glucose administration.

Two phases of insulin secretion are suggested by the portal vein IRI response to glucose. The early phase (between —1 and +3 min.) is followed by a nadir at

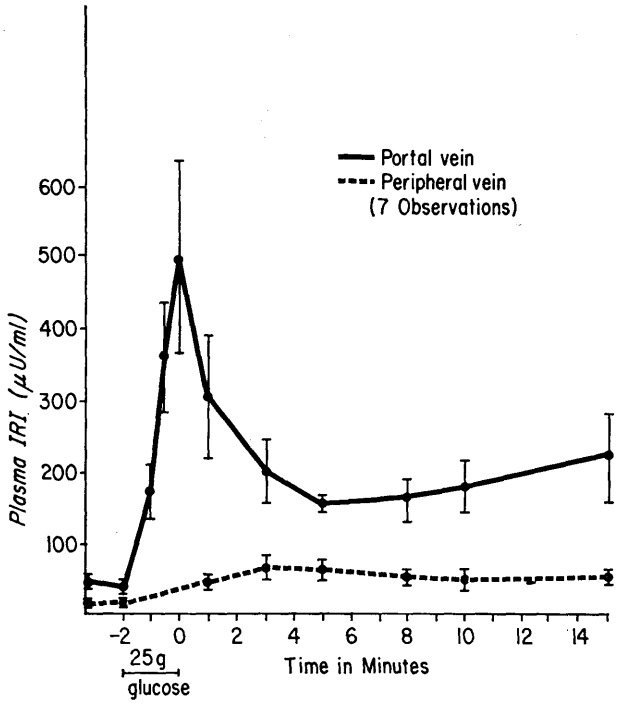

FIG. 1. Portal and peripheral vein IRI during and following a two-minute glucose infusion (25 gm.). Mean ± S.E.M. is shown.

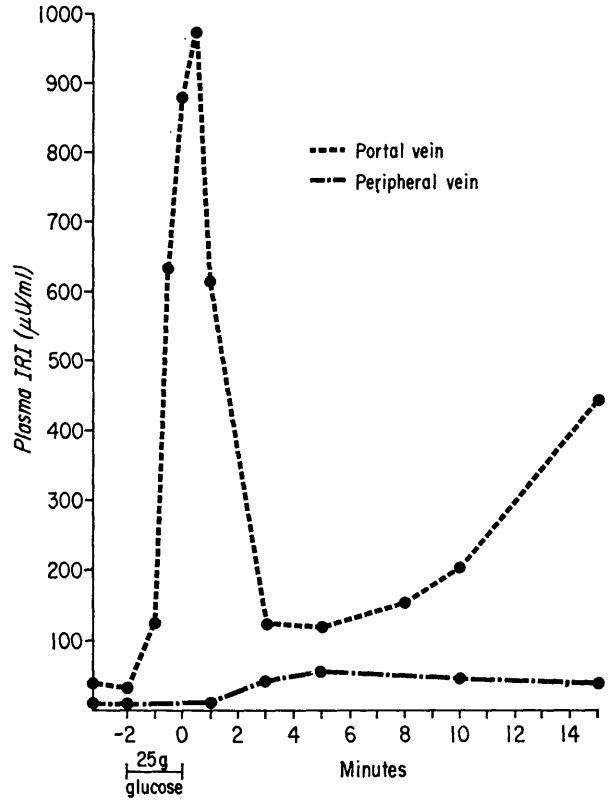

FIG. 2. Portal and peripheral vein IRI during and following a two-minute infusion of 25 gm. glucose in a single subject (E. G.).

five minutes when the portal and peripheral vein IRI concentrations reach their closest approximation. Portal vein IRI concentrations increase in the last three samples particularly at +15 min. Half of the subjects had greater portal vein IRI concentrations at fifteen minutes than at five minutes. In some individuals this second phase of insulin secretion was marked as can be seen in figure 2. Unfortunately, blood samples beyond fifteen minutes were not obtained.

A scattergram comparing portal vein and peripheral vein insulin responses is seen in figure 3. Insulin response curves for both the portal and peripheral veins were constructed and the integrated areas under the curves were assessed on all twelve subjects using values obtained at 1, 3, 5, 8, 10, and 15 min. after glucose infusion. A significant positive correlation between peripheral and portal vein insulin responses was observed ($r = +0.67$). The patient with the poorest correlation between peripheral and portal vein responses (E. G.) had obstructive jaundice due to choledocholithiasis.

Qualitative aspects of insulin secretion ("big" and "little" insulin) were studied to determine if the pattern varied during the early and later phase of insulin secretion. Figure 4 shows the results of IRI determinations on fractions from gel filtration when one and ten-minute portal vein plasma samples were placed on a 1 × 50 cm. Sephadex G-50 column. "Big" insulin is arbitrarily defined as IRI in fractions less than 30 per cent of the distance from the protein peak (albumin-I-131) to the salt peak (NaI-131). The absolute amount of "big" insulin was similar in both the one and ten-minute blood samples in the seven patients studied, but the proportion of "big" insulin was greater at ten minutes as a result of the lesser total amount of insulin.

Portal and peripheral vein blood glucose values during the above studies can be seen in table 2. Portal vein blood glucose is significantly higher than that in the peripheral vein after intravenous glucose administration. K values by the method of Amatuzio and coworkers[4] were determined using peripheral vein glucose values at three and fifteen minutes after completion of the glucose infusion. The mean k value was 4.2 ± 0.7. All but three of the k values were in the non-diabetic range (≧ 3.0). The three patients with k values less than 3.0 had normal oral glucose tolerance tests. Considering the stress of the experimental design

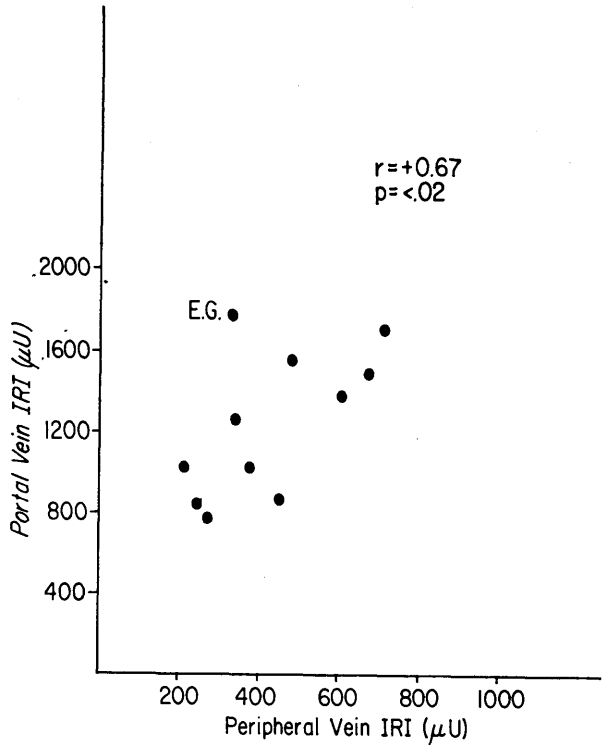

FIG. 3. Comparison of portal vein and peripheral vein insulin responses (area under the curve) following intravenous glucose. Curves were constructed for each response using IRI values obtained at 1, 3, 5, 8, 10, and 15 min. after glucose infusion and the areas under the curves were integrated.

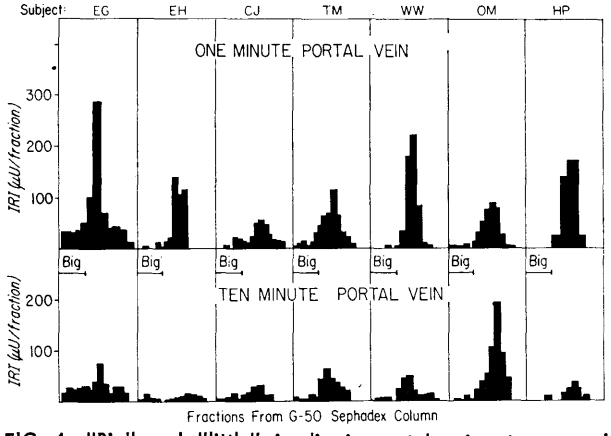

FIG. 4. "Big" and "little" insulin in portal vein at one and ten minutes following intravenous glucose administration in seven subjects. Dark bars represent IRI in fractions between the protein and salt peak when 1 ml. plasma is placed on G-50 Sephadex column. "Big" insulin is arbitrarily designated as described in Methods and the remainder of IRI is "little" insulin.

(preoperative status) and the short time period over which data for k values were accumulated, it is not surprising that three of the twelve values were abnormal. Although one of the subjects with a low k value (2.7) had the poorest insulin response to hyperglycemia, there was no significant correlation between insulin responses and k values.

TABLE 2

Portal vein and peripheral vein blood glucose before and after administration of 25 gm. glucose intravenously over two minutes

Time	Blood glucose (mg./100 ml.)* Portal vein	Peripheral vein
Baseline	93± 5.3	90± 5.0
Baseline	91± 4.7	87± 4.9
After glucose		
1 minute	317±14.2	263±16.5
3 minutes	281±11.0	240±14.6
5 minutes	260± 8.1	224± 9.2
8 minutes	236± 8.7	210± 9.0
10 minutes	226± 7.8	202± 6.9
15 minutes	210± 7.2	186± 9.8

*Mean ± S.E.M. is shown. Twelve observations.

DISCUSSION

A simple surgical technic involving catheterization of the portal vein via the collapsed umbilical vein has afforded an opportunity to compare peripheral and portal vein insulin concentrations following glucose infusion and to study the dynamics of insulin secretion in vivo. Curry, Bennett, and Grodsky have demonstrated two phases of insulin secretion in the isolated perfused rat pancreas.[5] A rapid secretory phase occurred first starting two minutes after onset of constant glucose perfusion and lasting for approximately two minutes. A slower secretory phase began at five to seven minutes and accelerated until termination of the sixty-minute perfusion. The second phase, partially inhibited by puromycin, was thought in part to be due to release of newly synthesized insulin. A remarkably similar insulin response was observed in vivo in the present study in response to a short two-minute glucose infusion in humans. One minute after the glucose infusion had started, portal vein insulin levels rose, reaching peak concentrations at completion of the two-minute infusion (zero time). The insulin values fell rapidly to a nadir three to five minutes after completion of the infusion, and then started to rise slowly again until termination of the study. The secondary phase of insulin secretion was observed clearly in only half of the twelve subjects. A longer period of blood sampling and continuous glucose infusion might have made the secondary phase of insulin secretion obvious in all subjects. Also in a closed system such as the present

experimental design necessitates, insulin recirculation tends to obscure the nadir between the two secretory phases. Although the second phase of insulin secretion may represent release of newly synthesized insulin, the absolute amount of "big" insulin in the portal vein during the early part of this secretory phase (at ten minutes) was no greater than that during the first phase of insulin secretion at one minute.

The delay in achievement of peak insulin concentrations at the periphery (two to five minutes after peak portal vein insulin concentrations) plus the lack of a diphasic response in peripheral insulin values such as seen in the portal vein indicate that peripheral insulin concentrations may not reflect accurately insulin secretory patterns. The failure of the peripheral vein insulin response curve to mimic that of the portal vein may be attributed largely to insulin trapping by the liver. Previous investigations have indicated that as much as 40 to 50 per cent of portal vein insulin may be extracted by the liver in a single transhepatic circulation.[6,7] Nevertheless, peripheral vein insulin concentrations may be used as a rough index of pancreatic insulin secretion, at least in the nondiabetic, since a significant positive correlation exists between portal vein and peripheral vein insulin responses to a glucose infusion. Whether a similar correlation exists between portal and peripheral vein insulin responses in diabetes and which of the two phases of insulin secretion is affected most by the diabetic state are questions which remain to be answered.

In certain conditions greater or lesser quantities of insulin may be trapped or degraded in the liver such as in our patient, E. G., who had the poorest correlation between peripheral and portal vein IRI response. This patient had obstructive jaundice and was the only subject with significant liver impairment. The possibility that greater amounts of insulin might be trapped in the liver and never reach the periphery in the diabetic must be considered. Others have postulated that derangement of the early phase of insulin secretion may be one of the first detectable abnormalities in diabetics.[8] Future studies are projected to evaluate diabetic patients when such patients requiring portography become available.

ACKNOWLEDGMENT

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