Insulin Production Rate Following Glucose Ingestion Estimated by Splanchnic C-Peptide Output in Normal Man

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Summary. Insulin production rate has been estimated in healthy male volunteers (n = 16), and evaluated with respect to splanchnic glucose exchange. Insulin production rate was calculated from splanchnic immunoreactive C-peptide output. C-peptide secretion was estimated by the hepatic venous catheter technique both in the basal state and for 2 h following ingestion of various glucose loads (0, 12.5, 25, 50, 75, and 100 g). The results demonstrate a basal insulin production rate of 0.017 ± 0.002 U/min (mean $\pm$ SEM) or 2.04 U/2 h. Values rose in a dose dependent manner from 2.6 ± 1.1 U/2 h after ingestion of 12.5 g of glucose to 10.8 ± 1.1 U/2 h following a glucose load of 100 g. Insulin retention by the liver was estimated at 0.012 ± 0.001 U/min in the basal state, and ranged from 47–85% ($70 \pm 2\%$) of production following an oral glucose load. It was also demonstrated 1) that the relative splanchnic glucose output was inversely related to the amount of ingested glucose, and reached a minimum when glucose in excess of 50 g was ingested; and 2) that hepatic glucose retention was directly proportional to insulin production rate ($r = 0.83$; $p < 0.001$; $n = 15$). It is suggested that the adaptive capacity of the splanchnic bed to retain glucose depending on the amount of ingested glucose guarantees that splanchnic glucose output fluctuates in healthy man only within a narrow range.

Key words: Insulin production rate, splanchnic C-peptide output, hepatic insulin retention, splanchnic metabolism, man, glucose.

Pancreatic insulin production has been studied previously in man by estimating the metabolic clearance of insulin [1, 2]. The valid use of radioactive tracer for this purpose has been questioned, since it is required

that the kinetics of labelled and unlabelled hormone be the same [1]. Therefore unlabelled insulin has been used to study the kinetics of insulin [3]. However, direct measurements of the splanchnic output of immunoreactive insulin (IRI) are of no value in determining pancreatic insulin production rate (IPR), since a large fraction of insulin is extracted by the liver on first pass [4–10]. In contrast to the latter observation it has been shown that C-peptide almost completely escapes the splanchnic bed [11, 12], and that C-peptide and insulin are secreted by the B-cell in equimolar concentrations [13].

The present study was therefore designed to calculate the pancreatic insulin production rate from the difference in hepatic venous and arterial immunoreactive C-peptide (C-peptide) concentrations multiplied by the estimated hepatic plasma flow (EHPF) both in the basal state and following glucose ingestion. This approach was adopted since the determination of insulin and C-peptide is not feasible in the portal blood of healthy man. Insulin retention by the liver was determined as the difference between insulin production rate as calculated from C-peptide values and the splanchnic output of IRI. The effects of ingested glucose on splanchnic glucose output (SGO) were also investigated to evaluate the interrelationship between an oral glucose load, insulin production rate and the amount of glucose assimilated by the splanchnic bed.

Material and Methods

Subjects

Subjects were 16 healthy male volunteers, between 19 and 29 years of age. All were within 10 per cent of ideal body weight (based on Metropolitan Life Insurance Tables, 1959) and not taking any drugs at the time of the study. None was diabetic or gave a

Table 1. Basal concentrations of blood glucose, serum IRI and C-peptide in arterial blood (A) and hepatic venous (HV) effluent. Their splanchnic output was calculated individually by the product of the arterio-hepatic venous difference (HV-A) times the EHBF (estimated hepatic blood flow) for glucose, or the EHPF for IRI and C-peptide. Insulin production rate (mU/min) was estimated from splanchnic C-peptide output. Mean ± SEM (n = 16)

Time (min)		−30	−15	0
EHBF (ml/min)		1455±72	1372±73	1426±86
EHPF (ml/min)		830±41	784±42	813±50
Blood glucose	A	93± 2	92± 8	94± 2
(mg/100 ml)	HV	103± 3	102± 2	103± 2
	HV-A	10± 2	10± 1	9± 2
Splanchnic glucose output (mg/min)		139±18	125±12	135±27
IRI (pmol/l)	A	66± 6	65± 7	60± 6
	HV	100±11	97± 9	97± 8
	HV-A	34± 6	32± 5	37± 4
Splanchnic-IRI-output (pmol/min)		25.2±3.6	23.8±2.9	29.5±4.3
(mU/min)		3.5±0.5	3.3±0.4	4.1±0.6
C-peptide (pmol/l)	A	642±37	619±33	622±36
	HV	808±50	761±43	775±50
	HV-A	166±17	142±20	152±17
Splanchnic-C-peptide-output (pmol/min)		131±13	111±14	121±15
Insulin production rate (mU/min)		18± 2	15± 2	17± 2

history of liver disease. Liver function tests were normal. All had an oral glucose tolerance test before the study with capillary glucose values < 160 mg/100 ml at 60 min, < 140 mg/100 ml at 90 min, and < 120 mg/100 ml at 120 min. For at least three days prior to the study a diet rich in carbohydrates (250–300 g) was ingested by all subjects. The nature, purpose and possible risks involved in the study were carefully explained to all subjects prior to obtaining their voluntary consent to participate.

Procedures

The studies were performed in the morning after an overnight fast (12–14 h). The procedure employed for the hepatic venous and arterial catheterization was similar to that described previously [14] with catheters inserted percutaneously into a peripheral vein, a femoral artery and through a femoral vein into a right sided hepatic vein under fluoroscopic control. After the catheters were in place arterial and hepatic venous blood was drawn at 7.5 min intervals both for a 30 min basal control period and for two h after oral glucose administration. Oral glucose (glucose monohydrate 55 g/130 ml corresponding to 50 g glucose, Boehringer-Mannheim, FRG) was ingested over three minutes after the control period followed by 100 ml of plain water. Glucose loads of 0 (n = 1), 12.5 g (n = 3), 25 g (n = 3), 50 g (n = 3), 75 g (n = 1), and 100 g (n = 5) were administered.

Glucose was analyzed in whole blood using the hexokinase reaction [15]. Serum concentrations of insulin [16] and C-peptide (Byk-Mallinckrodt) were determined radioimmunologically. The calculated interassay sensitivity (2 SD, n = 20) and coefficient of variation (1 SD; V%; n = 20) for the estimation of insulin was 1.7 μU/ml (V = 8.5%) for 10 μU/ml, 5.6 μU/ml (V = 7.6%) for 37 μU/ml, and 30.7 μU/ml (V = 8.6%) for 174 μU/ml. The calculated sensitivity for the C-peptide radioimmunoassay (n = 20)

was 0.17 ng/ml (V = 5.3%) for 1.7 ng/ml. Values for insulin (1 μU/ml equalling 7.3 pmol/l) and C-peptide (1 ng/ml equalling 331 pmol/l) in tables and figures were expressed in molarities.

The methods estimating splanchnic exchange of glucose by means of the hepatic venous catheter technique using indocyanine green dye [17] and the constant infusion technique [18] for splanchnic blood and plasma flow measurements have been described recently [14]. Splanchnic glucose retention (g/2 h) was calculated as the amount of ingested glucose minus splanchnic glucose output above basal per 2 h.

Splanchnic insulin (nmol/2 h) and C-peptide (nmol/2 h) output were calculated from their respective difference in hepatic venous and arterial concentrations multiplied by the estimated hepatic plasma flow (EHPF). Insulin production rate (IPR, units/2 h) was calculated from splanchnic C-peptide output assuming an equimolar release of C-peptide and insulin by the B-cell [13], and neglecting the minimal C-peptide retention by the liver [11, 12]. The estimation of IPR refers to the international insulin standard (1 mg = 24 IU), and to the molecular weight of human insulin (5734 Dalton [19]) and of human C-peptide (3021 Dalton [20]). Insulin retention by the liver was determined as the difference between the IPR and splanchnic-IRI-output.

Data in the text and figures are presented as the mean ± SEM. The unpaired Student t-test and calculation of the coefficient of correlation were employed for statistical analysis.

Results

Basal Concentrations in Arterial Blood and Hepatic Venous Effluent, and Splanchnic Exchange of Blood Glucose, Serum IRI, and C-Peptide (Table 1)

Prior to glucose ingestion, values of splanchnic exchange of glucose, insulin and C-peptide were studied for 30 min. During this period mean (n = 16) arterial and hepatic venous values fluctuated only to a small extent. Ranges were for splanchnic glucose output 34 to 266 mg/min, for splanchnic insulin output 10.4 to 40.9 pmol/min, and for splanchnic C-peptide output 54.6 to 254 pmol/min. Basal insulin production rate as calculated from splanchnic C-peptide output was 7.5 to 35 mU/min (mean 17 ± 2 mU/ min).

Estimated Hepatic Plasma Flow, Arterial Concentrations of Glucose, Insulin and C-Peptide

EHPF increased 15 min after oral glucose ingestion in all groups and reached peak values of 11 to 49 per cent above basal at 15 to 37.5 min. A secondary increase in EHPF was observed at 120 min in subjects receiving 75 and 100 g of glucose.

The arterial blood glucose levels rose by 25 to 102 per cent following oral glucose ingestion reaching peak levels at 22.5 to 52.5 min. Arterial blood glucose returned to basal levels within two hours only in individuals receiving 12.5 g or 25 g of oral glucose. In subjects receiving 50 to 100 g of glucose arterial

Table 2. Estimated hepatic plasma flow (ml/min), and arterial concentrations of glucose (mg/100 ml), insulin (pmol/l) and C-peptide (pmol/l) in healthy male subjects ingesting 100 g glucose. Basel values represent the mean ± SEM of five observations in each subject during 30 min preceding glucose loading

Time (min)	Basal	15	30	45	60	75	90	105	120
EHPF (ml/min)	820±38	1209± 65	1087± 93	1219±235	1018± 96	933± 44	1035± 49	963± 64	1176±192
Arterial glucose (mg/100 ml)	91± 4	126± 5	142± 20	178± 16	176± 13	176± 9	173± 16	173± 27	155± 25
Arterial IRI (pmol/l)	77±15	298±109	486±160	603±203	589±182	748±203	734±196	603±153	501±167
Arterial C-peptide (pmol/l)	641±69	1410±245	2072±417	2598±579	2863±655	3250±695	3575±608	3413±467	3109±407

Table 3. Splanchnic glucose output, splanchnic retention of ingested glucose (SG-retention), splanchnic insulin output, and splanchnic C-peptide output over 120 min following oral glucose loading (12.5, 25, 50, 75 and 100 g). Insulin production rate (units) was estimated from total splanchnic C-peptide output. Splanchnic insulin retention was calculated as insulin production rate minus splanchnic insulin output. Basal values were calculated for 2 h from the mean of three to five observations in each subject during 30 min preceding glucose loading, and the mean of all data in one subject without glucose loading. Thus all values are presented as mean ± SEM per 2 h, p vs. preceding group (excluding 75 g). [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.0025$; [d] $p < 0.0005$; [e] p vs. 12.5 and 25 g; [f] above basal

n	Basal (16)	12.5 (3)	25 (3)	50 (3)	75 (1)	100 (5)
Splanchnic glucose output (g)	15.1 ±1.3	9.0 ±2.0[f]	10.0 ±3.8[f]	12.3 ± 1.3[f]	20[f]	24.7 ± 1.0[d,f]
Splanchnic glucose retention (g)	–	3.5 ±2.0	15.0 ±3.8[a]	38.0 ± 1.3[c]	55	75.3 ± 1.0[d]
Splanchnic insulin output (nmol)	3.36±0.29	3.34±1.5	5.6 ±2.0	13.2 ± 2.7[a]	21.9	21.8 ± 3.7[b,e]
(Units)	0.46±0.04	0.46±0.22	0.79±0.28	1.84± 0.38[a]	3.05	3.02± 0.51[b,e]
Splanchnic C-peptide output (nmol)	14.9 ±1.32	17.6 ±6.3	23.9 ±5.1	49.0 ±10	56.2	77.8 ±12.2[a,b,e]
Insulin production rate (Units)	2.04±0.24	2.6 ±1.1	3.2 ±0.7	6.8 ± 1.4	7.8	10.8 ± 1.7[a,b,e]
Splanchnic insulin retention (nmol)	11.3 ±0.9	14.4 ±5.8	18.3 ±3.6	35.8 ±10.8	34.3	56.0 ±10.8[b,e]
(Units)	1.56±0.12	2.0 ±0.8	2.6 ±0.5	4.9 ± 1.5	4.7	7.8 ± 1.5[b,e]

blood glucose was still above basal levels at the end of the observation period.

Arterial IRI rose as expected two- to ninefold depending upon the amount of ingested glucose. Peak levels of IRI were reached at 15 min when 12.5 g of glucose and at 75 min when 100 g of glucose were administered.

Serum C-peptide concentrations also rose immediately following glucose ingestion. C-peptide levels after different glucose loads reached at 30 to 90 min their respective peaks which were two- to fivefold above basal. At 120 min C-peptide levels were still two- to fourfold above their respective basal concentration. The data for EHPF, and arterial concentrations of glucose, IRI and C-peptide obtained in the group receiving 100 g glucose are shown in Table 2.

Splanchnic Insulin and Glucose Metabolism (Table 3)

The influence of an oral glucose load on splanchnic glucose output, splanchnic insulin and C-peptide out-

put, and the splanchnic retention of glucose and insulin are shown in Table 3. Basal splanchnic glucose output was 126 ± 11 mg/min, or 15.1 ± 1.3 g/2 h. The cumulative splanchnic glucose output above basal during the two hour observation period (g/2 h) rose from 9.0 ± 2.0 g (n = 3) following a glucose load of 12.5 g to 24.7 ± 1.0 g (n = 5) when 100 g of glucose were administered (p < 0.0005). Conversely, net splanchnic glucose retention rose from 3.5 ± 2.0 g to 75.3 ± 1.0 g per 2 h. The amount of glucose retained by the splanchnic bed rose significantly from dose to dose when compared with the value of the respective preceding group.

Splanchnic IRI output in the basal state was 3.84 ± 0.33 mU/min corresponding to 0.46 ± 0.04 units of immunoreactive insulin per 2 h. Splanchnic IRI output remained unchanged after 12.5 g of oral glucose, and rose upon ingestion of 100 g glucose to 3.02 ± 0.51 U/2 h (p < 0.01).

Splanchnic C-peptide output in the basal state was 14.9 ± 1.32 nmol/2 h. The cumulative splanchnic C-peptide output rose consistently during the two hour observation period from 17.6 ± 6.3 nmol/2 h
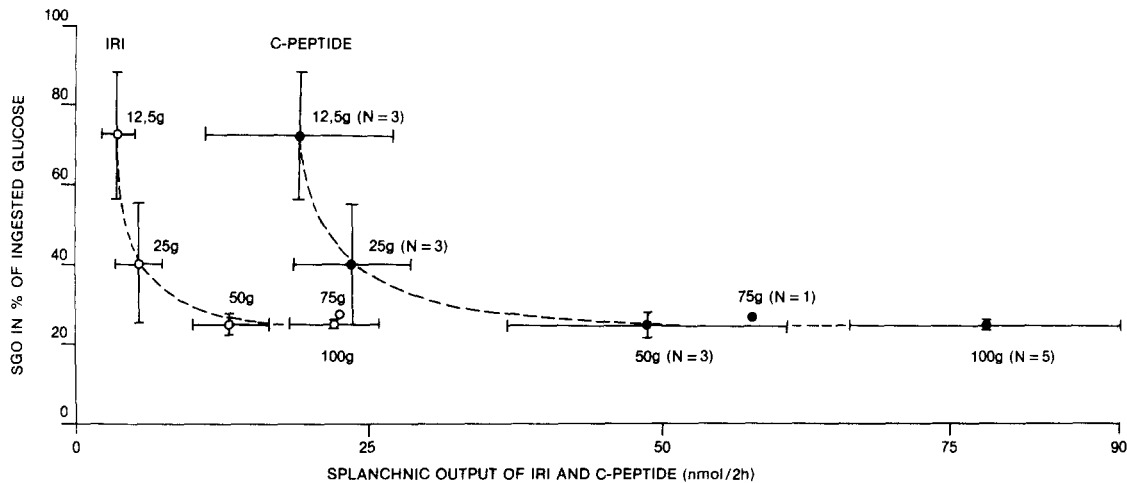
Fig. 1. Relation of splanchnic glucose output (SGO) in per cent of ingested glucose, and splanchnic output of IRI (o) and C-peptide (•) (nmol/2 h) in healthy male subjects. Glucose loads were 12.5 g (n = 3), 25 g (n = 3), 50 g (n = 3), 75 g (n = 1), and 100 g (n = 5). Values are given as mean ± SEM
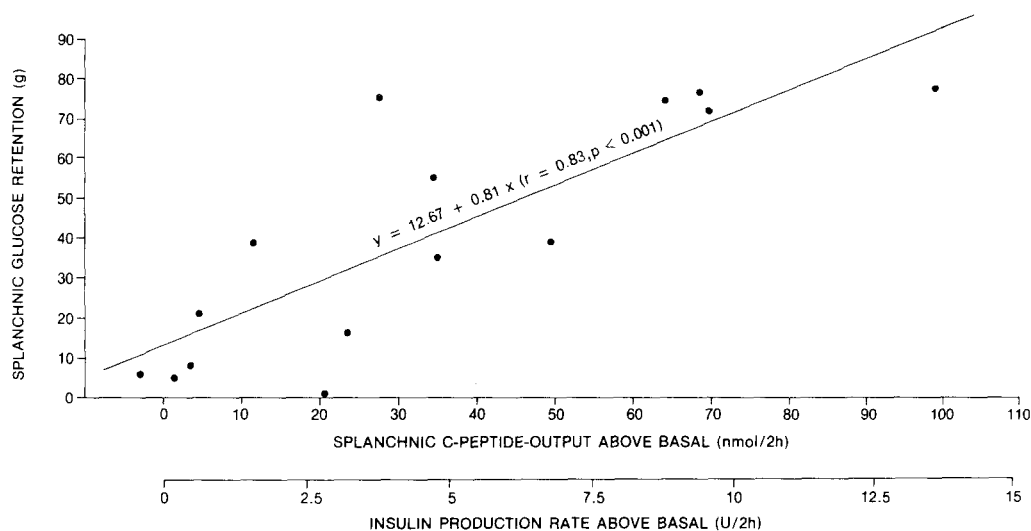

Fig. 2. Correlation between splanchnic glucose retention (g/2 h) and splanchnic C-peptide output above basal (nmol/2 h), and the insulin production rate above basal (units/2 h) in healthy male subjects (n = 15) ingesting various amounts of glucose. Glucose loads were 12.5 g (n = 3), 25 g (n = 3), 50 g (n = 3), 75 g (n = 1), and 100 g (n = 5)

(n = 3) following a glucose load of 12.5 g to 77.8 ± 12.2 nmol/2 h (n = 5) when 100 g of glucose were administered (p < 0.01).

Calculation of the insulin production rate from splanchnic C-peptide output on the basis of an equimolar release of C-peptide and insulin by the pancreatic B-cell revealed a basal production rate of insulin of 0.017 ± 0.002 U/min, or of 2.04 ± 0.24 U/ 2 h. Insulin production rate rose in a dose dependent manner from 2.6 ± 1.1 U/2 h after ingestion of 12.5 g of glucose to 10.8 ± 1.1 U/2 h following a glucose load of 100 g (p < 0.01).

Calculated splanchnic insulin retention in the

basal state was 11.3 ± 0.9 nmol/2 h corresponding to 1.56 ± 0.12 U/2 h. Splanchnic insulin retention rose to 14.4 ± 5.8 nmol/2 h after 12.5 g of oral glucose, and to 56.0 ± 10.8 nmol/2 h when 100 g of glucose were ingested. The relative amount of insulin retained by the splanchnic bed was 75 ± 2% during basal conditions, and 70 ± 3% (range 47–85%) following oral glucose ingestion.

The relation between splanchnic glucose output (SGO) in per cent of ingested glucose, and the splanchnic output of IRI and C-peptide in nmol/2 h is shown in Figure 1. It is apparent that the splanchnic output of IRI is well below that of C-peptide. It

can be seen that the relative SGO is high (72 ± 16 per cent) when a small dose of glucose (12.5 g) is administered. Relative SGO is however reduced when the oral dose of glucose is increased. The relative SGO reached its lowest value (25 ± 3 per cent) upon an oral glucose load of 50 g, and did not fall below 22 per cent even when the amount of ingested glucose was increased to 100 g. For the entire group of subjects ingesting different oral glucose loads a direct linear correlation ($r = 0.82$; $p < 0.001$; $n = 15$) was observed between splanchnic glucose retention and the insulin production rate above basal (Figure 2).

Discussion

In the present study the effect of different oral glucose loads on splanchnic glucose exchange, and insulin and C-peptide release have been examined in healthy postabsorptive volunteers. Specifically, the studies were designed to study the fate of insulin across the splanchnic bed, and to determine both the escape of glucose from the splanchnic bed and its retention by the liver (= splanchnic glucose retention) following different amounts of ingested glucose.

Most of the information available on insulin metabolism is based either on experiments utilizing labelled insulin from various animal species [21, 22, 23], or by studying the kinetics of the unlabelled hormone [3, 24].

Physiologically the cleavage of one molecule of proinsulin yields in the pancreatic B-cell one molecule of insulin and one molecule of connecting peptide. Both peptides are secreted into the portal blood in equimolar amounts [13, 25, 26], and consequently traverse the hepatic bed. In this course about half the amount of insulin presented to the hepatic bed is extracted by the liver [5, 10, 27]. In contrast to insulin, C-peptide [11, 12, 28] is removed only to a minor extent by the liver. The latter finding is based mainly on studies in rats [11, 28], whereas in lean normal weight pigs the reported hepatic extraction of C-peptide in the basal state was 19 ± 2 per cent [12]. In man studies of the metabolism of human C-peptide indicated that the kidneys are the major organ in C-peptide removal [29, 30].

However, the interrelationship of insulin and glucose metabolism in healthy man has been difficult to evaluate since direct measurements of insulin release into the portal blood are not readily available. Thus estimates of insulin release were obtained previously by using a model of the kinetics of insulin in man [3, 24]. By this means and assuming 47 per cent for the hepatic extraction of insulin the calculated daily basal

pancreatic production of insulin is 23.6 ± 7.7 units/ 1.73 m². This implies that the pancreas contributes 16.3 mU/min to the portal vein under basal conditions [3]. This estimate compares with a reported basal secretory delivery rate of insulin to the general circulation via the hepatic veins of 12 units [1], of 14 units [2], and of 4–13 units [31] over a 24 h period. Given a 75 ± 2 per cent hepatic extraction rate of insulin as observed in our study the values for insulin release as reported by others were in part higher than those reported by this study.

First suggestions to estimate endogenous insulin production by determination of C-peptide levels were made by Rubenstein et al. [32]. Our investigations using this concept showed an insulin production rate of 17 ± 2 mU/min, which amounts to 24.5 ± 2.9 units/24 h in the basal state, assuming a constant secretory rate and that no C-peptide was retained by the liver [11, 12, 28]. This compares well with a calculated insulin production rate of 23.6 ± 7.7 units/ 24 h reported by Sherwin et al. [3]. The basal posthepatic release of immunoreactive insulin was 5.5 ± 0.48 units/24 h, corresponding to about 25 per cent of the IPR. This reflects an hepatic insulin extraction of 75 per cent (range 47–88 per cent) by healthy subjects in the basal state. Although this estimate of hepatic insulin extraction is somewhat greater than that observed by others [7, 8], it is still in the upper range of what has been reported experimentally [9]. It has however to be kept in mind that the estimates of the retention by the liver of insulin depend both on the precision of the calculated splanchnic insulin and C-peptide output, both of which rest on small differences in hepatic venous – arterial plasma concentrations. The proportion of insulin taken up by the liver of healthy subjects has also to be compared with the 40 per cent of endogenous insulin retained by the liver of patients with portacaval anastomoses [7]. Thus a high hepatic insulin uptake might even reflect a normal liver function.

Absolute amounts of insulin retained by the liver of our healthy volunteers rose in parallel with increasing glucose ingestion and insulin production rate. However, the mean extraction of insulin by the liver over the 2 hour period after an oral glucose load remained with one exception between 65 and 85%, independent of the amount of ingested glucose. This is contrary to the suggestion of Field [33] that a greater fraction of insulin is extracted as greater amounts of insulin are presented to the liver.

The basal splanchnic glucose output of 126 ± 7 mg/min or of 15.1 ± 1.3 g/2 h in this study compares favourably with 145 ± 11 mg/min reported by others [34]. Our data indicate that following an oral glucose load the relative amount of glucose released

by the liver ranges from 72 to 25% over the ensuing two hours depending on the amount of ingested glucose. The relative share of glucose released by the splanchnic bed was greatest following ingestion of 12.5 g of glucose, and smallest after an oral load in excess of 50 g glucose. Relative SGO did not fall below 22% even upon ingestion of 100 g of glucose which is consistent with the data of Felig et al. [34]. However, the relative share of ingested glucose retained by the splanchnic bed also rose with the size of the glucose load, reaching a maximum at an oral load of 50 g. Splanchnic retention of ingested glucose was directly related to the estimated insulin production rate above basal levels (Figure 2). This is of great interest, since it is well known that the liver is a major site of insulin action in the control of glucose homeostasis [34, 35]. Thus it is postulated that insulin is the signal for turning off glycogen breakdown and allowing glycogen synthesis to occur [35, 36, 37]. A glucose feedback on the liver has also been proposed, so that the correlation between the insulin production rate and the splanchnic retention of an oral glucose load could in part be related to different plasma glucose concentrations [38]. Similarily, it has been observed in normal man that in a state of acute insulin and glucagon deficiency hyperglycaemia per se can suppress splanchnic glucose output without inducing splanchnic glucose uptake [39]. Other studies in vivo however suggest that autoregulation of hepatic glucose output independent of insulin does not occur [35].

The main factor facilitating the adaptation of the splanchnic bed to retain a large share of an oral glucose load may be the direct delivery of insulin to the liver, with a higher concentration of insulin in portal as compared to peripheral blood [40]. Thus it has been observed in this study that even an eightfold rise in ingested glucose from 12.5 g to 100 g was only followed by an approximately 2.5 fold increase in SGO from 9.0 ± 2.0 g to 24.7 ± 1.0 g. These data suggest that the liver, as the primary site of insulin action for the disposal of ingested glucose, protects peripheral tissues from extreme fluctuations in blood glucose (a) by retaining a greater percentage of a large glucose load, and (b) by providing a bigger relative share of an oral glucose load to peripheral tissues when only a tiny amount of glucose is ingested. Further studies are needed to understand the implications of our findings to pathological states.

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