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Glucose ingestion in dogs alters the hepatic extraction of insulin. In vivo evidence for a relationship between biologic action and extraction of insulin.

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Research Article

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Glucose Ingestion in Dogs Alters the Hepatic Extraction of Insulin

IN VIVO EVIDENCE FOR A RELATIONSHIP BETWEEN BIOLOGIC ACTION AND EXTRACTION OF INSULIN

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ABSTRACT Oral glucose (25 g) fed to seven healthy, conscious dogs resulted in an increase in peripheral plasma glucose from 109±3 to 178±10 mg/dl. Concurrently serum insulin increased in the portal vein to levels approximately threefold greater than those in the periphery. Hepatic insulin delivery rose from 10.8±0.7 to 59.0±19.9 mU/min at 60 min, coincident with an increased hepatic insulin extraction from 3.3 to 41.4 mU/min (corresponding to an increase in hepatic extraction from 31±4 to 59±7%), both returning to basal at 3 h. In each animal there was a positive correlation between hepatic insulin delivery and extraction ($r = 0.80$, $P < 0.001$ for the seven experiments combined). These changes in hepatic insulin delivery and extraction after glucose feeding were correlated with changes in hepatic glucose metabolism associated with insulin action. As hepatic insulin extraction increased, hepatic glucose output declined, both parameters returning to basal levels by 3 h, indicating a negative correlation between hepatic insulin extraction and hepatic glucose output ($r = 0.63$, $P < 0.001$; $n = 7$).

The factors that mediate this marked and rapidly occurring increase in hepatic insulin extraction after oral glucose are unknown, and may include hepatic insulin delivery, glucose levels in the blood supplying the liver, factors related to increased hepatic blood flow, and gut factors released by oral glucose intake. The association of changes in hepatic insulin extraction in vivo with an insulin effect on the liver as measured by hepatic glucose output is consistent with in vitro observations relating insulin degradation to receptor binding.

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INTRODUCTION

After secretion from the pancreatic islets, insulin travels in the portal blood to the liver, where it binds to specific cell surface receptors (1, 2). The liver is an important site for the biologic action and degradation of insulin (3–9). The biologic effects of insulin are dependent on rate of secretion and binding to the insulin receptor, both of these processes being control points for the biologic action of this hormone. It is, however, also conceivable that uptake and/or degradation of insulin in the liver and elsewhere may represent another control point. Although attempts have been made to define the factors that influence the hepatic extraction of insulin, the results have been contradictory. There is no agreement about the regulation of hepatic insulin metabolism, the physiologic factors that influence this process, and the relationship between the hepatic action and metabolism of insulin. To evaluate these variables we studied the changes in hepatic metabolism of insulin that occur with glucose feeding in dogs.

METHODS

During laporotomy under barbiturate anesthesia, (sodium pentobarbital, diabutal, Diamond Laboratories, Inc., Des Moines, Iowa; 30 mg/kg i.v.) end sampling catheters were placed in the portal and hepatic veins of seven mongrel dogs (18–28 kg; mean weight 22±1.6 kg) according to the methods described by Cherrington et al. (10). The portal vein catheter was introduced via the spleen and secured in the portal vein just before its bifurcation into right and left branches in the porta hepatis. The hepatic vein catheter was inserted and

secured under direct vision into the left common hepatic vein. These catheters were burrowed subcutaneously, filled with a dilute solution of heparin in saline (1:100), and secured in a subcutaneous pouch until the day of experimentation. The dogs were then allowed to recover from surgery and were studied in the conscious state 17–21 d later, provided leukocyte counts and hematocrits were within normal limits, and body weight was maintained. On the day of study after an overnight fast under local anesthesia, a femoral artery catheter was inserted through a small branch of the left femoral artery into the common femoral artery. A peripheral venous infusion catheter was also inserted and the portal and hepatic vein catheters were exposed.

The following experimental protocol was used. Hepatic blood flow was measured by estimation of hepatic clearance of continuously infused indocyanine green (11). Each experiment consisted of a 2-h base-line period after which the dog was fed 25 g glucose. During the base line and for 6 h after feeding, samples were collected for measurements of insulin, glucose, and indocyanine green from femoral artery, portal vein, and hepatic vein at the time points indicated. Femoral artery and portal vein samples were collected simultaneously and the hepatic vein sample was collected 25 s later to allow for hepatic transit time (12). An additional two dogs were studied as controls over a 4-h period. Dogs were studied while standing in a harness in the upright position in a well ventilated laboratory with ambient temperatures maintained at 70°F. When studied in this manner dogs do not appear to be under significant stress and tolerate the studies well. Less than 15% of total blood volume was withdrawn in each experiment and was replaced with an infusion of normal saline via the peripheral infusion catheter. The position of all catheters was verified at autopsy on completion of each experiment.

Assay and chromatographic methods. Serum insulin was measured by a modification of the double antibody technique (13). Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio, model 23A). Indocyanine green was measured spectrophotometrically at 780 nm using a Beckman spectrophotometer (model 34 Beckman Instruments, Inc., Fullerton, Calif.).

Calculations Hepatic extraction for insulin was calculated at every sampling time as a percentage of hepatic hormone delivery according to the formula: hepatic extraction (%) = [(hepatic delivery rate − hepatic removal rate)/hepatic delivery rate] × 100. Hepatic delivery rate (mU/min) = a + b; where a = HA plasma flow × FA(IRI) and b = PV plasma flow × PV (IRI). Hepatic removal rate (mU/min) = HV plasma flow × HV (IRI), where HV plasma flow = PV + HA plasma flows. HA is the hepatic artery; FA, the femoral artery; PV, the portal vein, HV, the hepatic vein; IRI, immunoreactive insulin.

Hepatic glucose output was calculated by the AV difference method derived from the product of plasma glucose level and flow in each of the three vessels.

Statistical methods. All results are expressed as mean±SEM. The statistical significance between group means was assessed by Student's *t* tests. Differences of means were considered significant if P was <0.05.

RESULTS

Control experiments. Basal hepatic extraction of insulin measured every 30 min was constant over 4 h in the two control animals (mean 46.1±1.0%; range 41–50%; coefficient of variation 6.5%). The mean values (±SEM) and ranges for insulin and glucose levels encountered in the three vessels, and for the hepatic plasma flows and insulin and glucose balances across the liver following oral glucose are given in Table I. The mean peripheral glucose levels and portal and peripheral insulin levels following oral glucose are plotted in Fig. 1. Peripheral glucose levels increased from 109 to 178 mg/dl at 30 min returning to base line at 3 h. As expected, a biphasic insulin response was observed in the portal vein. It is noteworthy that insulin levels in the portal vein increased to a threefold greater extent than those in the periphery as assessed by both peak levels and area under the curve.

The changes in hepatic insulin delivery and extrac-

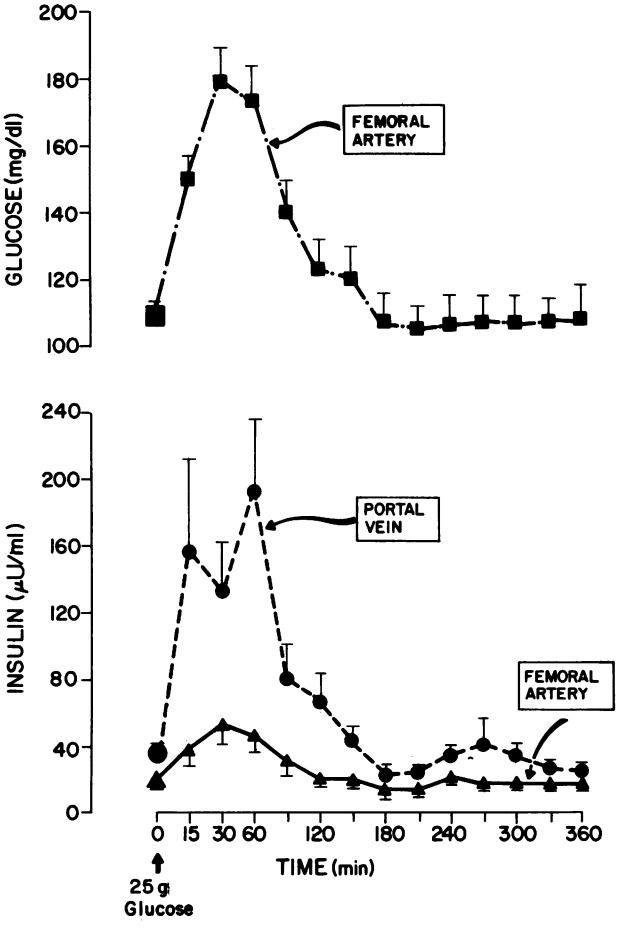


FIGURE 1 Peripheral glucose and portal and peripheral insulin levels in dogs (mean±SEM) following 25 g oral glucose (n = 7). The four measurements made during the base-line period were similar and are therefore measured to give the basal value at the time point zero.

TABLE I

Transhepatic Insulin and Glucose Balance before and after Oral Glucose (25 g) in Seven Dogs

	Basal	15	30	60	90	120	150	180	210	240	270	300	330	360
Femoral artery insulin, *μU/ml*	19±2	47±9	50±9	45±9	30±7	20±4	18±3	11±3	13±3	20±3	17±2	17±1	17±1	17±2
Portal vein insulin, *μU/ml*	37±4	156±56	139±26	192±54	81±21	66±18	43±9	22±7	23±5	33±8	41±15	34±8	26±3	25±3
Hepatic vein insulin, *μU/ml*	21±2	34±4	47±7	47±10	28±7	24±4	19±2	11±2	15±3	19±2	19±2	19±1	16±1	16±1
Hepatic artery plasma flow, *ml/min*	107±17	149±18	132±17	154±29	155±26	154±16	155±24	145±26	153±28	196±35	189±32	182±24	155±25	175±25
Portal vein plasma flow, *ml/min*	257±42	356±45	317±41	357±77	373±62	370±38	372±60	350±64	366±68	472±86	455±77	437±62	372±62	425±64
Insulin delivery to liver, *mU/min*	10.8±0.7	56.6±15.2	46.4±5.9	59±19.9	36.3±9.2	28.9±8.1	19.2±4.0	11.4±3.9	11.6±3.6	16.7±1.0	17.9±2.2	19.2±4.3	11.7±0.9	13.1±1.4
Insulin leaving liver, *mU/min*	7.5±0.7	17.4±2.7	19.1±1.7	17.6±2.3	14.8±3.0	13.1±2.5	10.4±2.3	6.6±1.8	8.5±2.2	11.8±1.2	11.5±1.2	10.9±2.0	8.4±0.9	9.4±1.2
Hepatic insulin extraction, %	31±4	60±8	55±6	59±7	51±8	46±7	41±7	35±6	26±3	29±7	33±9	35±9	28±6	28±5
Femoral artery glucose, *mg/dl*	109±3	150±7	178±10	173±12	139±9	123±9	120±10	112±9	107±6	107±6	107±5	107±5	106±5	107±6
Portal vein glucose, *mg/dl*	107±3	180±12	210±11	205±15	161±15	134±13	125±11	117±11	107±7	104±6	104±6	103±4	104±4	105±5
Hepatic vein glucose, *mg/dl*	120±5	178±11	203±12	189±15	153±12	135±12	128±9	121±9	117±7	117±7	114±6	116±6	115±5	116±7
Hepatic glucose output, *mg/kg/min*	2.00±0.30	1.52±0.60	0.26±0.52	−1.17±0.55	−0.67±0.45	−0.74±0.56	0.89±0.87	1.44±0.68	2.26±0.52	2.13±0.40	2.28±0.43	2.22±0.49	1.61±0.30	2.56±0.65

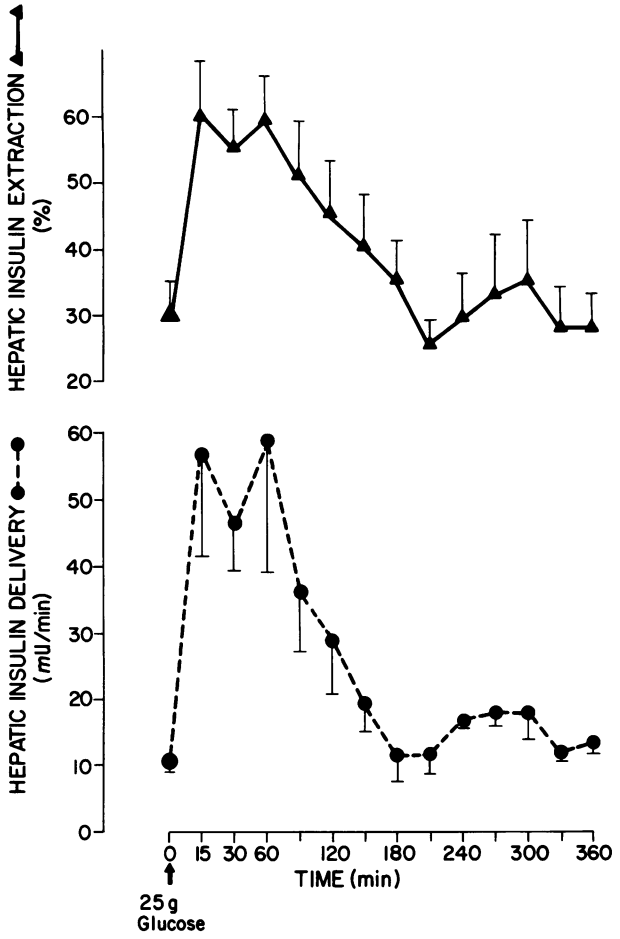


FIGURE 2 Hepatic insulin delivery and extraction (mean ±SEM) following oral glucose in dogs (n = 7). Zero time values were derived as in Fig. 2.

tion following glucose are illustrated in Fig. 2. Insulin delivery to the liver increased sixfold from a mean of 10.8 to 59 mU/min at 60 min, also exhibiting a biphasic profile, and returned to basal rates concomitant with the return to basal glucose levels at 3 h. Coincident with the increased hepatic insulin delivery, there was a marked increase in hepatic extraction of insulin from 3.3 to 41.4 mU/min, associated with a doubling in fractional insulin extraction from 30 to 60% returning to base line at 3 h. The profile of hepatic insulin extraction reflected the biphasic pattern of insulin secretion and hepatic delivery. The relationship between hepatic insulin delivery and extraction in each animal and the combined regression analysis of this relationship considering all dogs together is shown in Table II, with a representative plot from one animal in Fig. 3. It is evident that with increasing hepatic delivery there is a concomitant increase in hepatic insulin extraction ($P < 0.001$).

Following glucose feeding there was also a significant relationship between hepatic insulin and glucose fluxes. Thus as hepatic insulin extraction increased, hepatic glucose output declined and became negative as the liver began to take up glucose. This period of hepatic glucose uptake corresponded temporally to the increase in hepatic insulin extraction (Fig. 4). Concomitant with the fall in hepatic insulin extraction to basal levels, hepatic glucose output was restored to its fasting rate. The relationship between hepatic insulin extraction and hepatic glucose output in individual dogs and the combined regression analysis of this relationship in all seven animals is shown in Table III ($P < 0.001$) and a representative plot from one dog in Fig. 5.

TABLE II
Relationship of Hepatic Insulin Delivery (Abscissa) to Hepatic Insulin Extraction (Ordinate) in Seven Dogs

Dog	n	Slope	Intercept	r Value	P Value
1	13	2.07±0.27	−6.9±6.1	0.92	<0.001
2	14	0.77±0.15	31.2±5.1	0.82	<0.001
3	9	0.44±0.18	30.3±5.2	0.68	<0.05
4	10	0.60±0.13	28.3±3.4	0.85	<0.002
5	11	0.65±0.11	17.2±3.8	0.90	<0.001
6	14	0.86±0.28	8.06±6.0	0.67	<0.01
7	14	0.35±0.07	40.2±5.1	0.81	<0.001
Combined regression analysis*	85	0.48±0.05	‡	0.80	<0.001

* Represents the least squares estimate of the common slope in individual dogs by multiple regression analysis.
‡ With the multiple regression analysis separate intercepts were fitted for each dog. A common intercept was not considered statistically or biologically valid.

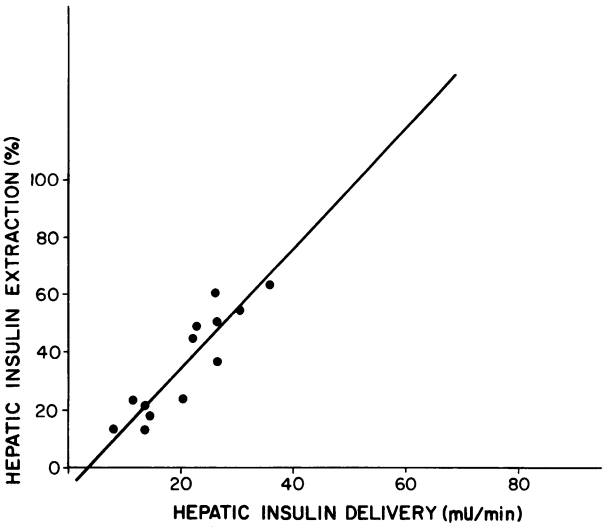


FIGURE 3 Relationship between hepatic delivery and extraction of insulin following oral glucose in a representative dog. $y = 2.07x - 6.9$; $n = 13$; $r = 0.92$; $P < 0.001$.

DISCUSSION

In the present experiments, we have demonstrated that following glucose ingestion, concomitant with the increased insulin delivery to the liver, there is a striking increase in hepatic insulin extraction. Both the increased hepatic insulin delivery and the increased hepatic insulin extraction are of limited duration, returning to base line 3 h after the glucose load. Furthermore, after glucose feeding, the increased hepatic insulin extraction observed is related temporally to a decrease in hepatic glucose output such that the

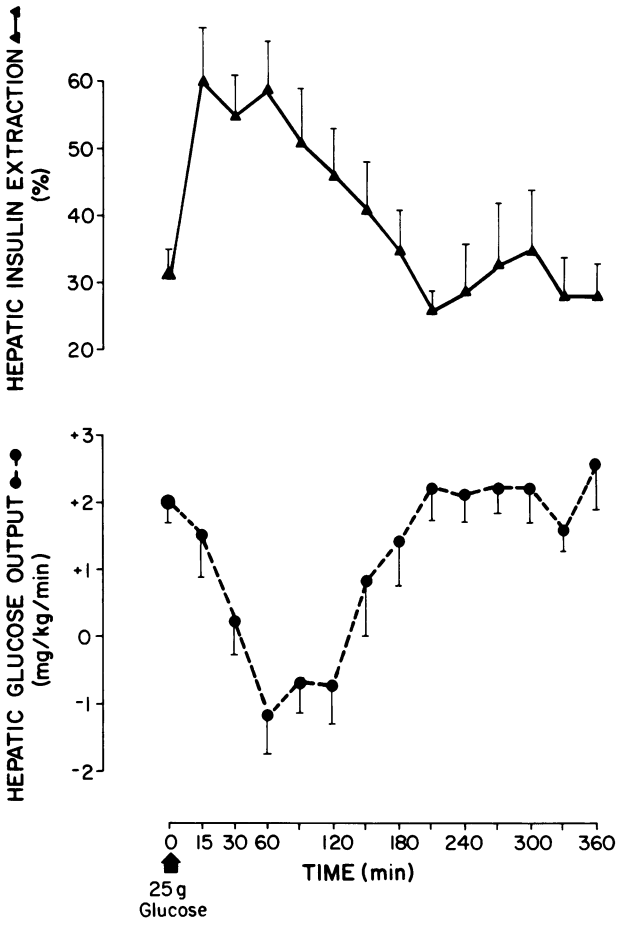


FIGURE 4 Hepatic insulin extraction and hepatic glucose output (mean±SEM) following oral glucose in dogs ($n = 7$). Zero time values derived as in Fig. 2.

TABLE III

Relationship of Hepatic Insulin Extraction (Abscissa) to Hepatic Glucose Output (Ordinate) in Seven Dogs

Dog	n	Slope	Intercept	r Value	P Value
1	13	−0.09±0.02	4.32±0.89	−0.79	<0.002
2	14	−0.09±0.02	5.28±1.51	−0.66	<0.01
3	9	−0.09±0.05	5.17±2.39	−0.54	<0.1
4	10	−0.09±0.02	5.10±1.14	−0.75	<0.002
5	11	−0.06±0.02	3.46±1.04	−0.60	<0.05
6	14	−0.03±0.02	1.88±5.46	−0.36	<0.2
7	14	−0.03±0.01	2.45±6.95	−0.57	<0.05
Combined regression analysis*	85	−0.06±0.01	‡	−0.63	<0.001

* Represents the least squares estimate of the common slope of individual dogs by multiple regression analysis.

‡ With the multiple regression analysis separate intercepts were fitted for each dog. A common intercept was not considered statistically or biologically valid.

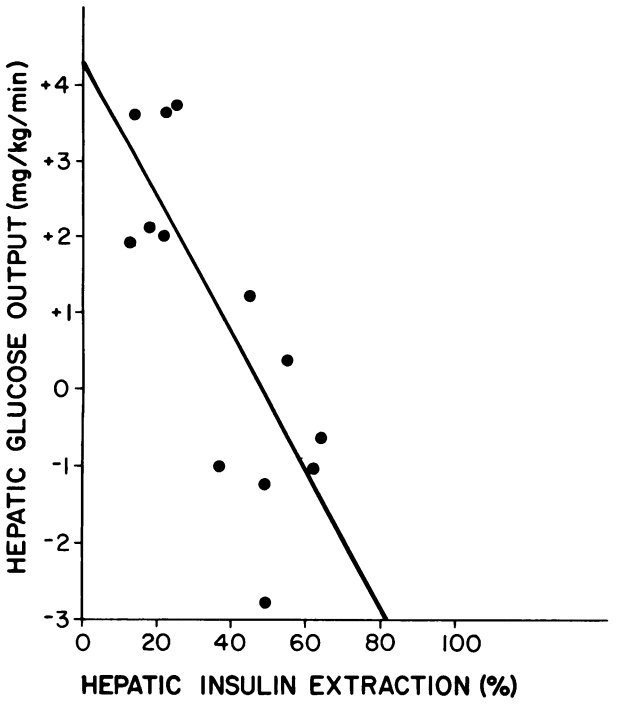


FIGURE 5 Relationship between hepatic extraction of insulin and hepatic glucose output following oral glucose in a single dog. The relationship in the other animals is shown in Table III. $y = -0.09x + 4.32$; $n = 13$; $r = 0.79$; $P < 0.002$.

period of maximal insulin action on the liver correlates with that of maximal insulin extraction.

Several investigators have studied hepatic insulin metabolism in animals and man. These studies have reached essentially the same conclusion, that the liver extracts 30–60% of the insulin delivered to it in a single passage (1, 3, 4, 7, 9). The regulation of this process and its significance in terms of controlling insulin economy in the body are, however, unknown. An understanding of the mechanisms whereby hepatic insulin extraction increases and returns to base line after oral glucose may provide unique insights into the physiological importance of hepatic insulin metabolism. After oral glucose, complex changes in gastrointestinal and pancreatic hormones, metabolites, and splanchnic blood flow occur. Each of these may exert an important influence on hepatic insulin extraction. It is uncertain whether this alteration in hepatic insulin extraction is related to the quantity of insulin reaching the liver, the portal vein insulin concentration itself, the increased portal vein flow that accompanies glucose absorption, an effect of blood glucose per se, or other factors associated with ingestion of glucose. Speculation into these factors can, however, set the direction for further studies related to the regulation of this important process.

Glucose itself is an important candidate as a possible regulator of hepatic insulin metabolism. Kaden et al. (9) and Waddell and Sussman (14) showed that after intraduodenal glucose administration in dogs, hepatic insulin extraction increased. On the other hand, Honey and Price (15) and Tranberg (16) found that glucose administration reduced the fraction of insulin extraction by the liver and Waldhausl et al. (17) and Olefsky et al. (18) reported no effects of glucose on insulin disposal. Although animals species, experimental models, and other factors were not comparable in these studies, it is possible that an important variable may be the route of glucose administration. Thus, as yet unidentified gut factors may mediate the alterations in hepatic insulin extraction after oral glucose. A possible effect of blood glucose levels on hepatic insulin extraction has not been assessed directly because in all studies using administration of oral or intravenous glucose, concomitant changes in hepatic insulin delivery, hepatic blood flow and, in the case of oral glucose, gut factors occurred. Assessment of hepatic insulin extraction under conditions of glucose clamping at incremental levels, and comparison of oral and intravenous glucose loads will be required to separate the effects of glucose from that of other factors in regulating hepatic insulin extraction.

Other potentially important factors that may determine the observed changes in hepatic insulin metabolism are the absolute quantity of insulin reaching the liver and the route of insulin delivery to the liver. As mentioned above, Kaden et al. (9) showed that the increase in hepatic insulin delivery occurring after oral glucose is associated with an increase in hepatic insulin extraction. Röjdmark et al. (19, 20), on the other hand, found that increases in hepatic insulin delivery induced by intraportal insulin infusion or insulin stimulation by arginine plus cholecystokinin-pancreozymin were associated with a reduction in hepatic insulin extraction. Mondon et al. (6) reported that with *in situ* perfusion of rat liver, the rate of hepatic insulin removal was proportional to arterial insulin concentration over a range of 20 to 500 μU/ml, although above this concentration, hepatic removal processes became saturated. Their data also indicated that the time-course of hepatic insulin uptake consisted of two distinct phases; an initial rapid phase, suggested to be associated with insulin binding to hepatic receptors, followed by a sustained rate of insulin removal thought to represent insulin internalization and degradation. Rubenstein et al. (7) showed saturation of insulin extraction in isolated perfused rat liver at perfusate concentrations > 2,000 μU/ml and similar conclusions can

be reached from the work of others (4, 6, 9, 16, 21). Although there is still uncertainty regarding the plasma insulin level at which hepatic insulin extraction is saturated, there is general agreement that saturation of this process does not occur at levels in the physiologic range such as after meals. The data reported here, consistent with that of others (9, 14, 22, 23), indicate that at subsaturation rates of hepatic insulin extraction, the fractional extraction of insulin delivered to the liver can be increased in the face of higher portal vein levels and hence hepatic delivery rates of insulin.

Other studies have suggested that the capacity of the liver to extract insulin may depend on the route of insulin delivery. Tranberg et al. (16) reported a 50% decrease in plasma clearance of insulin in association with a 10-fold increase in intraportal insulin concentration in man, although a reduction in insulin clearance was not seen with similar increases in peripheral insulin. Similarly, Goriya (24) described increased insulin clearance with portal, but not with peripheral insulin administration given over longer periods in dogs. On the other hand, insulin extraction was unchanged over a wide range of portal insulin concentrations following intraportal insulin infusion in dogs (12) and in hyperinsulinemic man (25).

The question of whether changes in hepatic blood flow might exert an influence on hepatic insulin extraction must be considered. In these studies, following glucose, hepatic plasma flow increased approximately twofold from a mean of 340 to 688 ml/min; most of this increment can reasonably be attributed to portal vein increases. Although this question has not been addressed directly in vivo, Karakash et al. (26) showed that insulin extraction by perfused rat liver remained constant despite doubling of the perfusion rate. On the other hand, Misbin et al. (21), using a similar system, showed that hepatic insulin clearance depended in part on the portal flow rate. In addition, we have obtained preliminary data that indicate that the hepatic extraction of glucagon tends to decrease after oral glucose despite the increase in portal blood flow, suggesting that portal flow is not a major determinant of hepatic peptide hormone extraction.

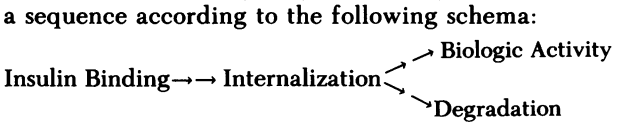
The relationship between receptor binding and extraction of insulin by the liver has been addressed by a number of investigators. Initially, it was believed that they were separate processes. Thus, Freychet et al. (27) reported that insulin binding and degradation by rat liver membranes were independent processes. An alternative concept was that insulin action was mediated by binding to one set of receptors and degradation by binding to an independent set of receptors. The suggestion that prior receptor binding might be a major factor in insulin degradation was first made by Terris and Steiner (28, 29). In in vitro studies, they showed a correlation between receptor binding and degradation in isolated rat hepatocytes and between retention and degradation of insulin in the perfused rat liver and suggested that insulin degradation is preceded by receptor binding. Similar observations were made by Olefsky et al. (30) with isolated rat hepatocytes and rat liver membranes.

In vitro studies comparing the biologic activity, degradation and receptor binding of insulin, proinsulin, C-peptide, and various insulin analogues such as desalanine insulin have provided interesting results. Proinsulin, which has 5–10% of the biologic activity of insulin (31), is removed by the isolated perfused rat liver 10–15 times less rapidly than insulin (7). These figures correlate with the known binding of proinsulin to hepatic insulin receptors, which is 5–10% of that for insulin (32). C-peptide, which exerts no biologic activity in the liver, is not metabolized by this organ (33, 34). These relationships, recently reviewed by Kitabchi (35), suggest that hepatic receptor binding and biologic activity of insulin-related peptides are proportional to their hepatic removal. This author also suggested that hepatic receptor binding affinity, biologic activity, and extraction appear to be related phenomena. Further support for this concept is provided by the recent work of Minaker et al. (36) who documented reduced insulin clearance in patients with cellular insulin resistance. Their data suggested that when insulin action is impaired owing to receptor or postreceptor defects, there is a corresponding decrease in insulin clearance. In this regard, Hruska et al. (37) have observed linkage between biologic activity and extraction of peptides other than insulin at the hepatic level. They showed that parathyroid hormone influences hepatic glucose output and is degraded by the liver, whereas the carboxyl terminal fragment of parathyroid hormone, which has no effect on hepatic glucose output, is not degraded by the liver. The data from the present experiments are consistent with the postulate that hepatic insulin extraction may be related to the extent of insulin action. Thus, in the fasted state, when insulin levels are low and hepatic insulin action is relatively small, hepatic insulin extraction is lower. However, after oral glucose, coincident with increased hepatic insulin action, there is an increase in hepatic insulin removal.

Because there is convincing evidence that hepatic insulin extraction occurs through a receptor-mediated pathway after internalization (28, 29, 38–40) we must consider the possibility that hepatic insulin extraction may be modulated at the receptor level. In this context, our observations that insulin extraction increases rapidly following oral glucose is particularly noteworthy

because it is consistent with major changes at the receptor level, perhaps mediated by specific changes in glucose or insulin. An analysis of the mechanisms by which this may occur suggests a number of possibilities. Firstly, there may be an increase in the number of insulin receptors at the cell surface. Synthesis of new insulin receptors would appear to be an unlikely explanation in view of the short time frame (41–43). However, it has recently been demonstrated that in hepatocytes, in addition to the exposed surface pool of insulin receptors, there is a very large intracellular pool of receptors (44). It is thus conceivable that a rapid shuttling of insulin receptors from intracellular to surface sites may account for the observed increase in hepatic insulin extraction. Decreased degradation of receptor complexes is a theoretical possibility, but this has not been previously shown. Recruitment of insulin receptors from some intracellular location might conceivably occur. Although there is no precedent for this postulate, it is of interest that increased glucose transport units have been shown to be recruited from intracellular sites by insulin in isolated rat fat cells (45, 46). Although these observations have no bearing on insulin receptors themselves, they do represent a mechanism whereby an increase in functional units may be achieved. A second mechanism involves oscillation by recycling of insulin receptors as demonstrated by Marshall et al. (47) in isolated rat adipocytes. Under appropriate circumstances, increased shuttling of insulin receptors could explain the rapid increase in the available number of insulin receptors at the cell surface at any given time. Thirdly, changes in receptor affinity represent an important mechanism that may underlie these rapid changes. Insel et al. (48) have shown that acute short-term changes in insulin receptor affinity occurred within 5 h of hyperglycemia or hyperinsulinemia in man and other recent reports have documented acute insulin receptor affinity changes associated with fasting (41, 49, 50), exercise (51), and oral glucose ingestion (52).

In conclusion, although there has been some confusion regarding the effect of glucose ingestion on hepatic insulin extraction, our data clearly indicate a rapid increase in hepatic insulin extraction related to increased insulin delivery to the liver following oral glucose. Concomitant changes in hepatic glucose output were observed. Whether these changes were coincidently or causally related has not been conclusively established. However, they raise the possibility (39) that in vitro they may be causally implicated in a sequence according to the following schema:



This study did not however, evaluate hepatic insulin receptor binding and there are, of course, numerous steps between receptor binding at the cell surface, internalization, and the biologic action of insulin. Therefore, the evidence presented in support of such a relationship is at present circumstantial. In vitro degradation of insulin may not be the same as in vivo uptake or extraction, and for these and other reasons, comparison between in vitro and in vivo experiments must be evaluated with caution. Additional in vitro and in vivo studies are therefore necessary to focus more directly on each of these parameters and their possible interrelationships. The physiology of hepatic insulin metabolism can probably only be fully appreciated when studied in relationship to simultaneous changes in hepatic insulin delivery, receptor status, and glucose uptake. The present results clearly indicate that hepatic insulin extraction in vivo is not constant and are consistent with previous suggestions that the liver is an important site for the regulation of insulin extraction, thereby controlling peripheral insulin delivery.

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