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Research Article

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Infusion of glucose at 25 mg/kg per min for 20 min resulted in a fivefold increase in arterial insulin levels and in reversal of splanchnic glucose balance to a net uptake. Splanchnic uptake of alanine, glycine, phenylalanine, lactate, and pyruvate fell by 30-60% due to a reduction in fractional extraction of these substrates, inasmuch as their arterial concentrations did not decline.

Administration of glucose at 2 mg/kg per min for 45 min resulted in a 19 mg/100 ml increase in arterial glucose concentration and a doubling of arterial insulin levels. Despite the small increment in insulin, hepatic glucose production fell by 85%. Splanchnic exchange of amino acids, lactate, and pyruvate was unaltered. Estimated total glucose utilization during the infusion was no greater than in the basal state, indicating lack of stimulation of peripheral glucose uptake.

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Influence of Endogenous Insulin Secretion on Splanchnic Glucose and Amino Acid Metabolism in Man

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ABSTRACT Splanchnic exchange of glucose, 20 individual amino acids, lactate, and pyruvate was studied in normal subjects in the postabsorptive state and after stimulation of endogenous insulin secretion by infusion of glucose at two dose levels. In the basal state, mean splanchnic glucose production was 3.4 mg/kg per min. A net uptake of lactate, pyruvate, and nine amino acids was observed, with alanine accounting for half of the total splanchnic-amino acid extraction.

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Administration of glucose at 2 mg/kg per min for 45 min resulted in a 19 mg/100 ml increase in arterial glucose concentration and a doubling of arterial insulin levels. Despite the small increment in insulin, hepatic glucose production fell by 85%. Splanchnic exchange of amino acids, lactate, and pyruvate was unaltered. Estimated total glucose utilization during the infusion was no greater than in the basal state, indicating lack of stimulation of peripheral glucose uptake.

It is concluded that: (*a*) inhibition of hepatic glucose production associated with glucose infusion and large increments in insulin levels occurs in the absence of a decrease in the concentration of circulating gluconeogenic substrate, suggesting an hepatic rather than peripheral effect; (*b*) the liver is the primary target organ whereby glucose homeostasis is achieved with small increments in insulin; (*c*) the relatively greater sensitivity of the liver's response to insulin as compared with an effect of insulin on the peripheral tissues, may be a consequence of the higher levels of endogenous insulin in portal as compared with peripheral blood.

INTRODUCTION

The central role of the liver in blood glucose homeostasis has long been recognized. Equally well established is the fact that insulin influences blood glucose levels not only by augmenting peripheral glucose utilization but also by inhibiting endogenous glucose production by the liver (1, 2). Two aspects of the relation of insulin to hepatic glucose metabolism, however, have not been directly examined in man: (*a*) does insulin inhibit gluconeogenesis by a direct action on the liver (3), or alternatively, by diminishing substrate supply, particularly amino acids (4); and (*b*) to what extent does diminished hepatic glucose production rather than augmented peripheral glucose utilization contribute to the over-all effect of small increments in circulating insulin. The importance of the former question is underscored by the recent observation in prolonged fasted man that hepatic gluconeogenesis is regulated by substrate availability (5). On the other hand, the evidence of diminished responsiveness to insulin in circumstances such as obesity (6) and aging (7), and of possibly increased effectiveness of insulin in prediabetes (8), necessitates identification of the relative importance of the liver as the target organ of insulin action.

In the present study, the hepatic venous catheter technique has been employed to determine splanchnic glucose and amino acid balance in normal subjects after stimulation of endogenous insulin secretion by infusion of glucose. Two dose levels of glucose eliciting large

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and small increments in circulating insulin, were employed. The importance of altered substrate presentation in insulin-modulated inhibition of gluconeogenesis was investigated by infusing a glucose load (25 mg/kg per min, "high dose") known to result in reversal of basal net hepatic glucose output (9) and in stimulation of circulating insulin levels comparable to those observed postprandially. In addition, a "low" glucose load (2 mg/kg per min) was administered to evaluate the sensitivity of hepatic glucose production to small increments in circulating insulin.

METHODS

Subjects

High dose glucose infusion. Six patients were studied while undergoing elective cardiac catheterization for diagnostic purposes (Table I). None had a history or evidence of right heart failure or primary liver disease. Liver function was considered normal on the basis of serum direct and indirect bilirubin, alkaline phosphatase, glutamic-oxaloacetic transaminase, and lactic dehydrogenase. The patients were informed of the nature, purpose, and risks of the research procedure to be performed (hepatic vein catheterization) in addition to the usual diagnostic studies, and gave their voluntary consent.

Low dose glucose infusion. The subjects were six healthy adult male volunteers who were employed by the Stockholm fire department. None had a history of diabetes or liver disease. Data for age, height, and weight are given in Table I. The nature, purpose, and possible risks involved in the catheterization procedure were fully explained to the subjects before obtaining their voluntary consent.

Catheterization, glucose infusion, and blood flow

The studies were performed in the morning after an overnight fast (12–14 hr). Teflon catheters with an outer diameter of 1.2 mm were inserted percutaneously into a brachial artery, and the right antecubital vein. A Goodale-Lubin catheter (No. 7 or 8) was inserted in an exposed left antecubital vein and advanced to the right hepatic vein under fluoroscopic control. The catheter tip was placed 3–4 cm from the wedge position. The catheter position was checked repeatedly by fluoroscopy before and during the infusion period. The catheters were kept patent by intermittent flushing with saline, and in the case of the hepatic venous catheter, by flushing with 0.5% sodium citrate in isotonic saline. Heparin was not used during the study.

After the catheters were in place, simultaneous arterial and hepatic venous blood samples were obtained for chemical analyses and hepatic blood flow during a 30-min control period, before glucose infusion. In the high dose study, glucose was administered intravenously at a rate of 25 mg/kg per min for 20 min. The infusate was prepared by dissolving the total amount of glucose to be delivered in 200 ml of normal saline. Blood samples were obtained at the conclusion of the infusion and 10 min after its termination. In the low dose study, glucose (as a 5% solution), was infused intravenously at a rate of 2 mg/kg per min for 45 min. Blood samples were obtained every 7–8 min after the start of the infusion.

Cardiac output was determined by the dye dilution technique after intravenous injection of 5 mg of Indocyanine green. Hepatic blood flow was estimated by the continuous infusion technique (10) employing Indocyanine green dye (11), in subjects K. M. and C. D. of the high dose group, and in all of the subjects in the low dose group. In subjects J. G. and C. E. of the high dose group, hepatic blood flow was determined in the basal state only (before glucose infusion), by the Indocyanine green plasma disappearance method (11).

TABLE I
Clinical Data on Subjects

Subject	Age	Sex	Height	Weight	Cardiac diagnosis
	yr		*cm*	*kg*	
High dose glucose infusion					
C. D.	39	M	173	70	Normal (systolic murmur)
K. M.	20	F	157	49	Mitral stenosis
J. G.	21	M	180	68	Mitral incompetence
C. E.	43	F	169	58	Normal (systolic murmur)
F. L.	34	F	157	66	Combined mitral lesion
A. H.	55	F	158	60	Normal (systolic murmur)
Low dose glucose infusion					
H. M.	26	M	180	67	Healthy volunteer
C. G.	26	M	184	71	Healthy volunteer
S. L.	36	M	174	67	Healthy volunteer
T. S.	28	M	178	72	Healthy volunteer
M. K.	21	M	192	80	Healthy volunteer
R. R.	25	M	172	65	Healthy volunteer

Blood analyses

Blood glucose was determined by the glucose oxidase procedure (12). Lactate (13) and pyruvate (14) were measured in whole blood by enzymatic techniques. Individual amino acids were determined by the automated ion-exchange chromatographic technique (15) on heparinized plasma deproteinized with sulfosalicyclic acid (16). Amino acid analysis was performed on the basal arterial and hepatic venous samples and on the specimens obtained at the conclusion of the high dose (20 min), and low dose (45 min) glucose infusions. Serum insulin was measured by immunoassay using talc to separate bound and free insulin (17). The paired *t* test and calculation of the coefficient of correlation were employed in the statistical analyses (18).

RESULTS

Splanchnic metabolism in the basal state. Splanchnic balance of glucose, lactate, pyruvate, and individual amino acids in the fasting state are shown in Tables II and III for the subjects in the high dose study, and in Tables IV and V for the subjects in the low dose study. Before glucose infusion, net release of glucose by the splanchnic circulation was demonstrable in all subjects. The basal rate of splanchnic glucose production was calculated from A–V differences and hepatic blood flow for the subjects in the low dose study, in all of whom flow measurements were available (Table IV). The observed mean value of 3.4 mg/kg per min is in close agreement with that reported by Bondy, James, and Farrar (9) who noted a mean rate of glucose production of 3.5 mg/kg per min in the fasting

TABLE II

*Arterial Insulin Concentration and Arterial Concentration and Splanchnic Exchange of Glucose, Lactate, and Pyruvate in the Fasting State and after High Dose (25 mg/kg/min) Glucose Infusion**

Subject		EHBF‡	Arterial insulin	Glucose		Lactate		Pyruvate	
				Arterial	A-HV§	Arterial	A-HV§	Arterial	A-HV§
		ml/min	*μU/ml*	*mg/100 ml*		*mmole/liter*		*mmole/liter*	
C. D.	Fasting	1100	7	86‖	−7‖	0.91	0.13	0.120	0.056
	20 min	1050	32	339	+25	1.00	−0.15	0.154	−0.078
	30 min	—	27	252	+4	1.06	−0.37	0.145	−0.125
K. M.	Fasting	985	8	76	−11	0.35	0.11	0.101	0.016
	20 min	900	49	264	+23	0.36	0.08	0.108	−0.006
	30 min	—	43	210	−3	0.32	−0.04	0.112	−0.167
J. G.	Fasting	800	10	72	−19	0.47	0.04	0.079	−0.002
	20 min	—	39	251	+5	0.58	−0.04	0.076	−0.039
	30 min	—	46	205	−9	0.68	−0.18	0.058	−0.032
C. E.	Fasting	1050	9	86	−17	0.60	0.18	0.179	0.081
	20 min	—	58	334	+34	0.72	0.05	0.255	−0.101
	30 min	—	52	212	+1	0.74	−0.25	0.380	−0.153
F. L.	Fasting	680¶	18	85	−9	0.36	0.25	0.063	0.031
	20 min	—	112	290	+20	0.38	0.22	0.080	0.028
	30 min	—	125	243	+8	0.48	0.16	0.098	0.006
A. H.	Fasting	950¶	12	71	−6	0.32	0.18	0.070	0.046
	20 min	—	45	273	+21	0.28	0.16	0.087	0.039
	30 min	—	42	213	−3	0.30	0.20	0.121	0.021
Mean values									
	Fasting	928	10.7	79.3	−11.5	0.50	0.15	0.102	0.038
	20 min	—	54.5	291.8	+21.3	0.55	0.05	0.127	−0.026
	30 min	—	55.8	222.5	−0.3	0.60	−0.08	0.152	−0.075

* The glucose was infused for 20 min. The 20-min specimens were drawn at the conclusion of the glucose infusion and the 30-min specimens were drawn 10 min after completion of the infusion.

‡ Estimated hepatic blood flow.

§ Arterio-hepatic venous difference.

‖ Fasting values for glucose in each subject represent the mean of two sets of simultaneous arterial and hepatic venous samples.

¶ Estimated at 20% of cardiac output (31).

state. A consistently positive arterio-hepatic venous difference, indicating net uptake was observed for lactate and pyruvate (Tables II and IV). Of the 20 amino acids studied, significant uptakes were demonstrable for 9: alanine, glycine, serine, threonine, valine, tyrosine, phenylalanine, methionine, and α-aminobutyrate (Tables III and V). In accordance with previous studies (5), splanchnic uptake of alanine exceeded that of all other amino acids, accounting for 47–48% of total splanchnic extraction of amino acids. A consistent output was observed only for citrulline.

Response to high dose glucose infusion. Infusion of glucose at a rate of 25 mg/kg per min resulted in a rise of 180–250 mg/100 ml in arterial glucose concentration, a fivefold increment in peripheral insulin levels, and a consistent reversal of hepatic glucose output to a net uptake (Fig. 1, Table II). Despite an increment in arterial concentrations of lactate and pyruvate, their extraction by the splanchnic bed fell significantly ($P < 0.05$) after glucose infusion (Table II).

In Table III the arterio-hepatic venous differences for 20 individual amino acids after the high dose glucose infusion are compared with the values in the basal state. In association with the reversal in hepatic glucose output and with high levels of circulating insulin, consistent declines in the splanchnic uptake of alanine, glycine, phenylalanine, valine, and α-aminobutyrate were observed. In the case of alanine, glycine, and phenylalanine, this diminution was due to a reduction in the fractional extraction of these amino acids, since the arterial concentrations were not significantly altered. Thus, the arterio-venous difference for alanine, the pri-

mary gluconeogenic substrate taken up by the liver, fell by 32% due to a significant reduction in the fractional extraction of this amino acid (Fig. 2). In a like manner, fractional extraction of glycine and phenylalanine fell by 50–60% below basal levels (Table III). Although in four of the subjects one cannot exclude the possibility of an increase in hepatic blood flow to account for these changes in A–V differences, it is noteworthy, that no increase in hepatic blood flow was observed in the two subjects (C. D. and K. M.) in whom pre- and postglucose measurements were obtained (Table II). Furthermore, were altered blood flow responsible for the changes observed in splanchnic amino acid extraction, the exchange of all amino acids should be equally affected. It is thus of interest, that the uptake of threonine, serine, methionine, and tyrosine, and the output of citrulline did not decline significantly after the high dose glucose infusion.

In accordance with previous studies (19, 20), stimulation of a fivefold increment in peripheral circulating-insulin levels resulted in a decline in the arterial concentration of valune, leucine, tyrosine, α-aminobutyrate, and threonine (Table III). Since the peak effect of insulin on peripheral release and arterial levels of amino acids occurs at 1 hr (20, 21), it is likely that a greater diminution, and possibly, decreases involving additional amino acids, would have been observed had blood samples been obtained at 60 min after initiation of the glucose infusion.

Response to low dose glucose infusion. The effect of intravenous administration of glucose at a rate of 2 mg/kg per min on arterial glucose and insulin levels, hepatic glucose production, and hepatic blood flow is shown in Table IV, in which the mean values for the six subjects studied are presented. The response of a representative subject (R. R.) is shown in Fig. 3. The low dose glucose infusion resulted in a maximal rise in mean arterial-glucose concentration of 19 mg/100 ml and in a doubling of peripheral insulin levels (Table IV). Particularly noteworthy is the fact that in association with these small increments in circulating glucose and insulin, mean splanchnic glucose output fell by 85%. Although hepatic blood flow fell by 12% during the course of the infusion, the primary factor responsible for the diminution in splanchnic glucose production was not the reduction in flow but the marked change in the A–V difference for glucose which fell from mean levels of − 16 to − 18 mg/100 ml in the basal state to − 2.9 mg/100 ml at the termination of the glucose infusion (Table IV). The relation between arterial glu-

TABLE III

Influence of High-Dose Glucose Infusion on Arterial Concentration and Arterio-Hepatic Venous Differences (A–HV) of Plasma Amino Acids

	Arterial concentration			A–HV		
	Basal	Postglucose*	P‡	Basal	Postglucose*	P‡
Taurine	50.5 ±2.9§	50.2 ±1.8		1.3 ±1.9	−0.50 ±2.0	
Threonine	128.8 ±10.3	118.2 ±11.2	<0.005	22.8 ±6.2	20.7 ±5.0	
Serine	126.3 ±7.6	120.8 ±8.5		29.6 ±7.8	25.0 ±4.0	
Proline	212.3 ±22.8	216.3 ±19.7		5.0 +4.5	1.8 +2.3	
Citrulline	29.8 ±2.5	29.7 ±2.9		−5.8 ±1.3	−7.8 ±2.9	
Glycine	228.3 ±21.6	212.2 ±30.7		39.5 ±5.3	14.5 ±4.6	<0.05
Alanine	288.5 ±39.1	265.0 ±39.2		117.3 ±16.0	79.3 ±7.0	<0.0125
α-aminobutyrate	22.5 ±4.7	19.8 ±4.9	<0.025	2.4 ±1.2	0.4 ±0.8	<0.01
Valine	229.2 ±12.5	215.1 ±14.9	<0.02	11.3 ±4.7	0.5 ±4.4	<0.05
½ Cystine	103.8 ±5.7	103.2 ±6.0		4.2 ±3.0	10.8 ±9.2	
Methionine	21.5 ±1.8	20.8 ±2.2		6.0 ±1.6	5.6 ±1.0	
Isoleucine	58.8 ±6.2	56.0 ±6.2		5.2 ±4.4	0.80 ±2.5	
Leucine	114.8 ±12.6	107.7 ±13.7	<0.025	9.0 ±9.0	2.0 ±3.6	
Tyrosine	46.5 ±3.6	41.8 ±3.8	<0.05	10.7 ±2.1	6.8 ±1.1	
Phenylalanine	45.0 ±3.5	43.0 ±4.5		9.0 ±2.5	4.0 ±1.1	<0.05
Ornithine	72.5 ±7.7	76.7 ±4.5		−9.0 ±7.3	2.0 ±1.3	
Lysine	166.0 ±12.9	160.0 ±10.6		−0.7 ±15.6	10.8 ±9.8	
Histidine	73.8 ±3.5	72.8 ±4.0		4.0 ±2.3	1.2 ±2.1	
Tryptohpan	32.3 ±5.8	27.6 ±6.6		−0.2 ±1.9	1.2 ±3.6	
Arginine	50.8 ±4.7	50.6 ±4.8		4.0 +7.0	9.8 ±1.7	

* Postglucose samples were obtained at the conclusion of a 20-min infusion of glucose at a rate of 25 mg/kg per min.

‡ *P*, probability that changes in arterial concentration and A–HV after glucose are a change occurrence (paried *t* test).

§ Mean ±SE, micromoles per liter.

TABLE IV

Influence of Low-Dose Glucose Infusion (2 mg/kg per min) on Arterial Levels of Insulin and on Arterial Concentration and Splanchnic Exchange of Glucose, Lactate, and Pyruvate

	Basal		Glucose infusion					
Time (min)	−15	0	7.5	15	22.5	30	37.5	45
Arterial glucose *mg/100 ml*	69.8 ±2.5*	69.3 ±3.4	78.7 ±2.8	81.5 ±3.8	84.8 ±3.3	86.5 ±3.2	87.8 ±3.8	88.7 ±4.0
			(<0.025)‡	(<0.025)	(<0.01)	(<0.005)	(<0.005)	(<0.005)
Arterial insulin *μU/ml*	8.9 ±1.4	8.3 ±1.9	—	16.4 ±4.9	—	15.0 ±4.9	—	17.7 ±5.5
				(<0.05)		(0.05 < P < .1)		(<0.05)
A–HV glucose *mg/100 ml*	−15.8 ±4.2	−17.6 ±2.5	−13.7 +5.6	−9.8 ±4.8	−6.3 ±3.7	−7.2 ±3.7	−5.1 +3.6	−2.9 ±1.4
				(<0.05)	(<0.02)	(<0.01)	(<0.005)	(<0.001)
Splanchnic glucose production *mg/kg per min*§	3.4 ±1.0	3.4 ±0.6	2.8 ±1.2	1.9 ±1.0	1.1 ±0.6	1.2 ±0.6	0.9 ±0.6	0.6 ±0.3
				(<0.025)	(<0.005)	(<0.005)	(<0.005)	(<0.005)
Arterial lactate *mmole/liter*	0.62 ±0.07	0.69 ±0.06	0.72 ±0.05	0.73 ±0.12	0.72 ±0.08	0.73 ±0.06	0.70 ±0.06	0.73 ±0.06
Splanchnic lactate uptake§ *mmole/min*	0.203 ±0.035	0.233 ±0.039	0.209 ±0.014	0.253 ±0.077	0.245 ±0.047	0.249 ±0.028	0.231 ±0.039	0.286 ±0.068
Arterial pyruvate *mmole/liter*	0.067 ±0.003	0.069 ±0.007	0.064 ±0.005	0.066 ±0.007	0.064 ±0.007	0.073 ±0.006	0.070 ±0.005	0.074 ±0.006
Splanchnic pyruvate uptake§ *mmole/min*	0.036 ±0.007	0.021 ±0.005	0.026 ±0.006	0.023 ±0.004	0.019 ±0.006	0.025 ±0.006	0.019 ±0.004	0.033 ±0.007
EHBF‖ *ml/min*	1319 ±95	1293 ±109	1235 ±90 (<0.05)	1196 ±113 (<0.05)	1167 ±81 (<0.05)	1191 ±88 (<0.05)	1143 ±67 (<0.05)	1177 ±76 (<0.025)

* Mean ±SE

‡ Probability that number differs from basal value by chance alone (paried *t* test).

§ Calculated from arterio-hepatic venous differences and estimated hepatic blood flow.

‖ Estimated hepatic blood flow.

TABLE V

*Arterial Concentration and Splanchnic Uptake of Amino Acids in the Basal State and after Low-Dose (2 mg/kg per min) Glucose Infusion**

	Arterial concentration		Splanchnic uptake‡	
	Basal	Postglucose	Basal	Postglucose
	micromole/liter		*micromole/min*	
Taurine	43.8 ±1.0§	41.6 ±1.0	0.7 ±1.3	1.0 ±0.6
Threonine	98.2 ±8.6	92.0 ±10.0	12.1 ±1.0	10.7 ±1.6
Serine	127.6 ±10.1	119.2 ±7.4	28.8 ±5.1	20.8 ±1.4
Proline	198.8 ±41.0	181.6 ±32.7	6.8 ±11.2	6.8 ±6.2
Citrulline	35.4 ±3.2	32.8 ±2.2	−10.8 ±4.2	−10.8 ±5.4
Glycine	181.8 ±17.3	178.0 ±21.3	5.8 ±2.8	7.7 ±3.0
Alanine	192.6 ±18.1	186.7 ±19.0	64.2 ±8.2	55.0 ±11.9
α-aminobutyrate	26.8 ±4.6	24.4 ±4.8	0.9 ±0.3	0.7 ±1.3
Valine	206.5 ±25.1	203.4 ±18.3	5.6 ±2.0	1.5 ±2.3
½ Cystine	91.8 ±3.7	91.0 ±9.0	−0.2 ±4.8	−7.9 ±11.6
Methionine	18.0 ±1.1	16.6 ±1.3	3.0 ±0.9	3.8 ±0.6
Isoleucine	58.3 ±6.7	49.2 ±5.0	−2.0 ±0.5	−1.9 ±1.9
Leucine	112.3 ±15.4	107.6 ±10.0	−4.3 ±0.7	−3.3 ±3.0
Tyrosine	46.5 ±3.8	42.8 ±3.0	7.7 ±1.8	6.0 ±0.9
Phenylalanine	47.5 ±2.3	43.0 ±2.6	4.5 ±0.9	3.0 ±0.6

* The basic amino acids were not measured in the low dose study.
‡ Calculated from arterio-hepatic venous differences and splanchnic plasma flow.
§ Mean ±SE.

cose, insulin, and splanchnic glucose output is underscored by the direct correlation between arterial glucose and insulin levels (Fig. 4), and the inverse correlation between arterial glucose concentration and hepatic glucose output (Fig. 4). In contrast to the response observed with large increments in circulating insulin (*vide supra*), there was no change in splanchnic uptake of lactate and pyruvate (Table IV), and of amino acids (Table V).

Since extrahepatic glucose production is negligible in postabsorptive man (22), with a constant arterial glucose concentration and in the absence of glycosuria, the rate of total peripheral glucose utilization must equal the rate of hepatic glucose production plus the rate of exogenous glucose infused. During the low-dose glucose infusion, arterial glucose concentration was stable for the last 15–22 min of the study and was well below the renal threshold for glycosuria (Table IV). Since the rate of glucose infusion was kept constant at 2 mg/kg per min, total glucose utilization may be estimated by adding 2.0 to the observed rate of hepatic glucose output (Table IV). In Table VI, the estimated rate of glucose utilization in the basal state is compared with that calculated for the latter part of the glucose infusion. Whereas hepatic glucose output declined significantly in

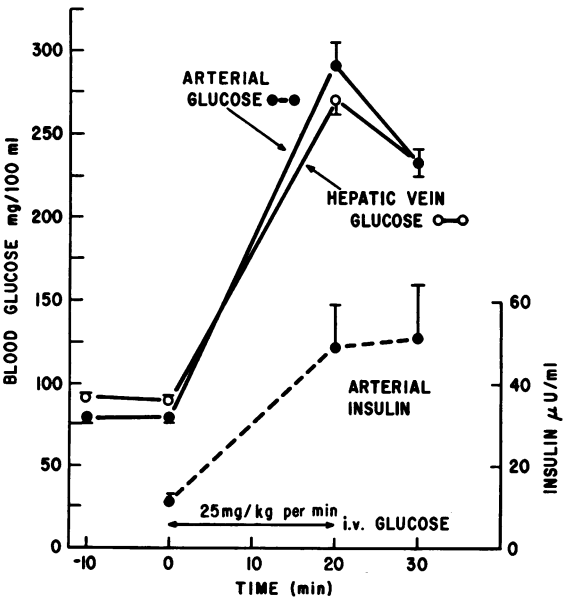

FIGURE 1 Arterial- and hepatic-venous blood glucose levels and arterial insulin concentration before and after infusion of glucose at 25 mg/kg per min. Mean values ±SE are shown. Insulin samples were not obtained between 0 and 20 min during which the initial insulin response and presumably peak insulin levels were observed.

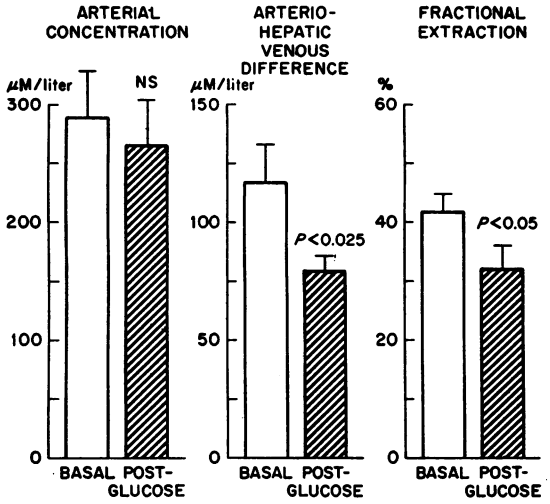

FIGURE 2 Arterial concentration and splanchnic extraction of plasma alanine in the basal state (open bars), and after infusion of glucose at 25 mg/kg per min for 20 min (cross-hatched bars). Lines at top of bars represent the standard error. *P* values are based on paired *t* test.

all subjects, a consistent increment in peripheral glucose utilization could not be demonstrated during the glucose infusion. The estimated rate of glucose utilization remained stable or decreased in five subjects and increased consistently above basal levels in only one subject (Table VI).

DISCUSSION

In the current study, the influence of large and small increments in insulin secretion on glucose and amino acid balance across the splanchnic bed has been directly examined in intact man. Endogenous, glucose-stimulated insulin secretion rather than exogenous insulin administration was chosen for study inasmuch as endogenous insulin is secreted directly into the portal circulation, resulting in portal-vein insulin concentrations which are 10-fold higher than peripheral levels (23). In attempting to determine if insulin regulates gluconeogenesis via direct action on the liver or via alterations in peripheral substrate supply, and in determining the relative importance of the liver and peripheral tissues in the glucoregulatory response to small increments in insulin, maintenance of physiologic portal–peripheral insulin gradients is of paramount importance. Simulation of the physiologic situation with exogenous insulin thus would necessitate injection of the insulin into the portal vein.

The findings in the present study in the basal state confirm previous observations indicating that alanine is quantitatively the primary amino acid extracted by the splanchnic circulation (5), accounting for half the total hepatic amino acid uptake. This primacy of alanine as gluconeogenic substrate appears in turn to be a consequence of the relatively greater rate at which alanine is released from peripheral muscle tissue as compared to other amino acids (24). In fact, fairly good agreement exists for most amino acids between their relative rates of splanchnic uptake (Tables III and V) and muscle output (24).

The response of splanchnic amino acid metabolism to large increments in endogenous insulin secretion is particularly noteworthy. After the high-dose glucose infusion, a significant uptake of glucose by the splanchnic circulation was demonstrable. In association with this complete reversal of net hepatic glucose production, the A–V differences for alanine, glycine, and phenylalanine were significantly reduced. This diminution in splanchnic uptake of gluconeogenic amino acids was not due to altered substrate supply, inasmuch as the arterial concentrations of these amino acids were unchanged. The decreased splanchnic uptake of glycogenic substrate in association with hyperinsulinemia was clearly a consequence of the 25–60% reduction in the fractional extraction of these amino acids. In a like manner, splanchnic uptake of lactate and pyruvate also declined significantly despite a slight increment in ar-

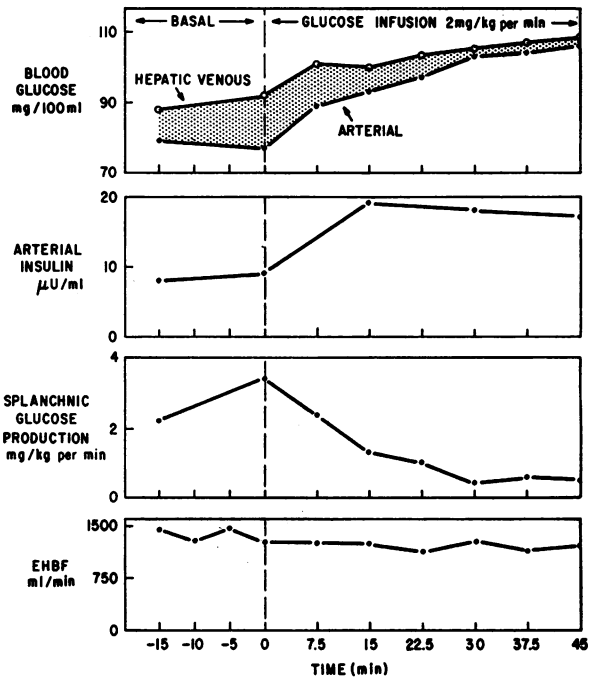

FIGURE 3 Arterial- and hepatic-venous glucose levels, arterial insulin concentration, splanchnic glucose production, and estimated hepatic blood flow (EHBF) in subject R. R., in the basal state and during infusion of glucose at 2 mg/kg per min.

terial concentrations. The data thus suggest that insulin-induced inhibition of gluconeogenesis in intact man is the result of a direct action on the liver rather than a consequence of insulin-mediated alterations in peripheral substrate supply. In accord with this conclusion is the observation that close intraarterial injection of insulin in the human forearm for a period of 26 min fails to diminish significantly the release of alanine from forearm muscle (21). Furthermore, the marked augmentation in gluconeogenesis observed in diabetic acidosis (25), occurs in the face of diminished arterial levels of alanine, glycine, threonine, and serine (26), suggesting a primary enhancement of hepatic amino acid extraction in the absence of adequate insulin. While there is no question that insulin reduces net amino acid release from muscle (21), thereby lowering the total concentration of circulating α-amino nitrogen (27), this effect involves primarily the branched-chain amino acids, leucine, isoleucine, and valine (20, 21). With respect to alanine and other glycogenic substrates, the data support an hepatic rather than peripheral effect for insulin. Nevertheless, the peripheral antilipolytic action of insulin may contribute to the observed hepatic effects by reducing the supply of free fatty acids necessary for gluconeogenesis. In addition, the supply of the glycogenic substrate glycerol may also be diminished by this peripheral action of insulin.

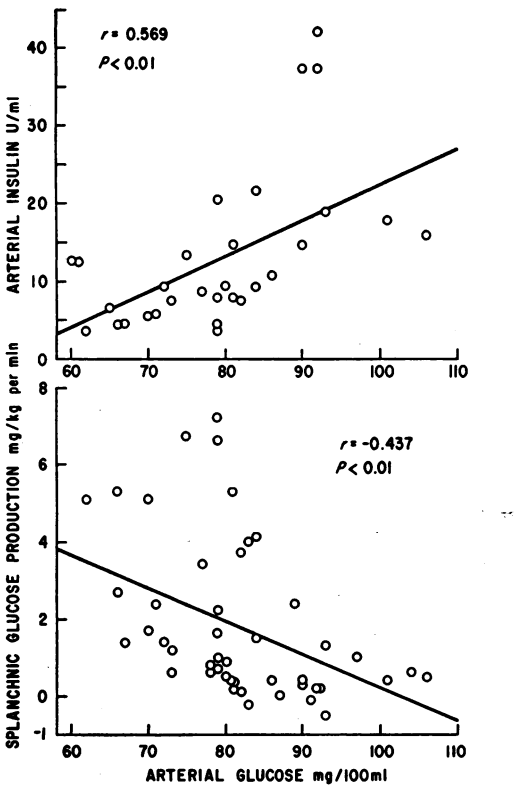

FIGURE 4 Relation of arterial glucose concentration to arterial insulin levels (upper figure) and to hepatic glucose production (lower figure) in subjects receiving the low-dose glucose infusion (2 mg/kg per min).

TABLE VI
*Influence of Low-Dose Glucose Infusion on Estimated Total Glucose Utilization**

	Estimated total glucose utilization				
	Basal‡	Glucose infusion			
	milligrams/kilograms per minute				
Time (min)	−15	0	30	37.5	45
Subject					
H. M.	6.7	5.3	6.1	6.0	3.5
C. G.	5.3	5.1	3.6	2.4	3.0
S. L.	—	2.0	2.3	1.9	1.8
T. S.	1.4	1.7	2.4	2.0	2.4
M. K.	1.4	2.7	2.5	2.4	2.1
R. R.	2.2	3.4	2.4	2.6	2.5
Mean	3.4	3.4	3.2	2.9	2.6
SE	±1.0	±0.6	±0.6	±0.6	±0.3

* Estimated total glucose utilization = splanchnic glucose production (Table IV) + glucose infused (2 mg/kg per min).
‡ In the basal state total glucose utilization is equal to splanchnic glucose production.

In the low dose study, an increase in insulin secretion resulting in no more than a doubling of mean peripheral-insulin concentration was accompanied by an 85% reduction in splanchnic glucose production. The relative sensitivity of the liver to the glucoregulatory action of insulin is underscored by the failure to observe a significant mean increment in peripheral glucose utilization during the course of the glucose infusion (Table VI). The latter finding is in accord with the observation that a doubling of basal arterial insulin levels fails to produce a consistent increase in glucose uptake by the superficial or deep tissues of the human forearm (21). The seemingly greater sensitivity of the liver need not necessarily reflect an inherently greater responsiveness on the part of the hepatocyte to a given insulin concentration, but may be a consequence of the higher ambient levels of endogenous insulin in portal as compared with peripheral blood (23). In either case the data indicate that in intact man in the postabsorptive state the liver rather than peripheral tissues is the primary target organ by means of which glucose homeostasis is achieved with small increments in circulating insulin. A similar conclusion was previously reached by Combes, Adams, Strickland, and Madison on the basis of observations in dogs with portacaval shunts (28).

In contrast to the high dose study, splanchnic uptake of alanine, lactate, and pyruvate failed to decline during

the low dose infusion. Since the rate of splanchnic uptake of these glucose precursors accounts for no more than 15% of net glucose production in the basal state (Table IV and V), the 85% reduction in glucose output may reflect an inhibition of glycogenolytic processes by small increments in insulin, with gluconeogenic mechanisms remaining intact.

Finally, the question may be raised as to whether the findings in the present study are in fact a consequence of endogenous insulin secretion or alternatively a direct effect of glucose on hepatic glucose production. That such is not the case is suggested by the high rates of hepatic glucose output observed in untreated, insulin-dependent diabetic subjects in whom arterial glucose concentration is markedly increased (25, 29). In addition, infusion of glucose at a rate of 2 mg/kg per min fails to reduce hepatic glucose production in insulin-dependent diabetic patients.[1] Furthermore, the studies of Ishiwata, Hetenyi, and Vranic (30) in which glucose infusion failed to inhibit hepatic glucose output in depancreatized dogs receiving a constant intraportal infusion of insulin sufficient to maintain the fasting blood glucose in the normal range, suggest that the role of insulin in hepatic glucose homeostasis is regulatory rather than permissive. Thus only the extra insulin normally evoked by a glucose load is capable of reducing hepatic glucose output. Nevertheless the possibility must be considered that factors other than insulin, such as inhibition of glucagon secretion, may contribute to the effects of glucose infusion observed in the present study.

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