Effect of Glucose, Independent of Changes in Insulin and Glucagon Secretion, on Alanine Metabolism in the Conscious Dog

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ABSTRACT To study the effects of hyperglycemia on the metabolism of alanine and lactate independent of changes in plasma insulin and glucagon, glucose was infused into five 36-h-fasted dogs along with somatostatin and constant replacement amounts of both insulin and glucagon. Hepatic uptakes of alanine and lactate were calculated using the arteriovenous difference technique. [^{14}C]Alanine was infused to measure the conversion of alanine and lactate into glucose. Hyperglycemia (Δ115 mg/dl) of 2 h duration caused the plasma alanine level to increase by over 50%. This change was caused by an increase in the inflow of alanine into plasma since the net hepatic uptake of the amino acid did not change. Taken together, the above findings indicate that glucose per se can significantly impair the fractional extraction of alanine by the liver. Hepatic extraction of lactate was also affected by hyperglycemia and had fallen to zero within 90 min of starting the glucose infusion. This fall was associated with a doubling of arterial lactate level. Conversion of [^{14}C]-alanine and [^{14}C]lactate into [^{14}C]glucose was suppressed by 60$\pm$11% after 2 h of hyperglycemia, and because this fall could not be entirely accounted for by decreased lactate extraction an inhibitory effect of glucose on gluconeogenesis within the liver is suggested. These studies indicate that the plasma glucose level per se can be an important determinant of the level of alanine and lactate in plasma as well as the rate at which they are converted to glucose.

INTRODUCTION

It has been generally accepted that alanine plays an important role in the transfer of nitrogen and carbon from extrahepatic tissues to the liver (1, 2). Although numerous studies have been carried out in vitro to investigate both the release of alanine by muscle (3–6) and the uptake of the amino acid by the liver (7–10), few such studies have been carried out in vivo. Chochinov et al. (11, 12) measured alanine flux in normal and diabetic subjects, while others have examined the effects of glucose infusion on splanchnic alanine uptake (13, 14) but it is clear that a systematic examination of the effects of the various factors (insulin, glucagon, and glucose) that might influence alanine flux in the whole animal has not been carried out.

Considerable evidence points toward a close relationship between the plasma glucose and alanine concentrations in vivo. The administration of large amounts of glucose, either intravenously (13) or orally (14), results in substantial increases in the plasma alanine concentration. In certain conditions, often associated with abnormal carbohydrate metabolism, such as obesity and Cushing's syndrome, pronounced elevations in the plasma concentration of alanine are observed (15, 16). Because the glycemic changes in the above states are associated with altered plasma levels of insulin and/or glucagon that might themselves affect alanine metabolism, (13, 14, 16) it has not been possible to deduce whether the changes in alanine concentration are caused by changes in the plasma levels of the pancreatic hormones, resistance to the actions of these hormones or secondary to changes in the plasma glucose concentration per se. Furthermore, it is not known whether the altered alanine levels are attributable to changes in the rate of appearance or disappearance of the amino acid from the vascular compartment.

Because the potential interaction between the pancreatic hormones and glucose in regulating the alanine concentration, the present studies were undertaken to examine the effect of hyperglycemia on total alanine flux and on alanine extraction by the liver. To define

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the effects of hyperglycemia independent of changes in insulin and/or glucagon secretion, the sugar was infused along with somatostatin (to inhibit the endocrine pancreas) and basal replacement amounts of insulin and glucagon. The results indicate that hyperglycemia per se exerts marked effects on alanine and lactate metabolism including the rate at which they are converted to glucose.

METHODS

Animals and surgical procedures. Experiments were carried out on 10 mongrel dogs (17–22 kg; average wt, 21.2 kg) of either sex which had been fed a high protein diet (43% carbohydrate, 25% protein; Wayne Dog Chow, Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) for 2–3 wk before use and which had been without food for 36 h. Catheters were inserted into a hepatic vein, the portal vein, a splenic vein, and a femoral artery under general anesthesia as described (17) at least 2 wk before experiment. On the day of study the catheters were exteriorized from their subcutaneous pockets under local anesthesia (2% Lidocaine; Astra Pharmaceutical Products, Inc., Framingham, Mass.) their contents were aspirated and saline was infused through them at a slow rate until the experiment was begun. Angiocaths (18 gauge; Abbott Diagnostics, Diagnostic Instruments, South Pasadena, Calif.) were then inserted percutaneously into the two cephalic veins and one saphenous vein. Blood was sampled from the arterial, portal, and hepatic vein catheters. Somatostatin was infused through one cephalic vein while indocyanine green and [^{14}C]alanine were given through the other. The pancreatic hormones, insulin and glucagon, were given through the splenic catheter and glucose or saline was infused through the saphenous vein. After completion of the preexperimental preparation the conscious dog was placed in a Pavlov harness where it stood calmly for 20–30 min before the start of an experiment.

On the day immediately preceding the experimental day, blood was withdrawn to determine the leukocyte count and hematocrit of the animal. Only dogs which had (*a*) a leukocyte count below 16,000 mm^3, (*b*) a hematocrit above 38%, (*c*) a good appetite (consuming at least two-thirds of daily ration), and (*d*) normal stools were used.

Experimental design. Each experiment consisted of a 90-min period of insulin titration, a 30-min control period, and a 120-min test period. At time −120 min, an infusion of somatostatin (0.8 μg/kg per min) was started to inhibit endogenous insulin and glucagon secretion (18) and the infusion of [^{14}C]-alanine was also begun. Intraportal replacement infusions of insulin (300 μU/kg per min) and glucagon (1.0 ng/kg per min) were started simultaneously with somatostatin. The plasma glucose level was then monitored every 5 min and the rate of insulin infusion was adjusted to maintain normoglycemia. The glucagon infusion rate was never altered. The maximum change required in the insulin infusion was 50 μU/kg per min and the final mean rate of insulin infusion was 286±23 μU/kg per min. The last alteration in the insulin infusion rate was made at least 30 min before the start of the control period. The infusion rates of both hormones then remained fixed throughout the remainder of the experiment so that during the test period the glucagon and insulin levels were basal and unchanging.

At time zero, two separate protocols were initiated. Five dogs were given a peripheral saline infusion while a second set of dogs were infused with glucose in the manner described previously (19) such that a step increment in plasma glucose concentration occurred.

Collection and processing of blood samples. The collection and processing of blood samples has been described elsewhere (18), as have the methods of immunoassay used for plasma insulin and glucagon (20, 21) and the methods of column chromatography used for the determination of the plasma [^{14}C]alanine- and [^{14}C]lactate-specific activities (18).

Materials. [U-^{14}C]alanine (New England Nuclear, Boston, Mass.) was used as the tracer (1 mCi/0.53 mg). By analysis using column chromatographic techniques it was determined that at least 99% of the counts infused were in alanine. Insulin and glucagon were purchased from Eli Lilly & Co., Indianapolis, Ind. Cyclic somatostatin was obtained from Bachem, Inc., Marina Del Rey, Calif. Indocyanine green was obtained from Hyson, Wescott and Dunning, Inc., Baltimore, Md. All hormone solutions were prepared with normal saline and contained 0.3% bovine albumin.

Calculations. The balance of various substrates (lactate, alanine, and glucose) across the liver, the splanchnic bed and the nonhepatic splanchnic tissues (henceforth referred to as "gut") were calculated as described (17). Similarly the conversion of circulating alanine and lactate to glucose was calculated in the manner reported (18). The rate of appearance (Ra)[1] and rate of disappearance (Rd) of alanine from the plasma were estimated by the method described for glucose (18). The method is based on a single compartment analysis in which it is assumed that rapid changes in the specific activity and concentrations do not occur uniformly within the pool. To compensate for nonuniform mixing, the nonsteady-state term of the equation was multiplied by a correction fact of 0.65. Since the volume distribution of alanine is not well defined, for calculation purposes we have assumed it to be equal to the volume of distribution of glucose. The volume of distribution for glucose was measured using an injection of [3-^{3}H]glucose at the beginning of each experiment as described (18).

RESULTS

Table I summarizes the combined control period alanine and lactate data from both experimental groups. It is evident that the portal vein specific activity of alanine was considerably lower (34%) than the specific activity of the amino acid in the artery. Because the ratio of the portal venous to arterial alanine specific activity was 0.66 and the ratio of the plasma alanine concentrations in the two vessels was 1.16, it is possible to calculate that about one-half of the dilution resulted from net addition of unlabeled alanine to the plasma and the remainder from exchange of labeled for unlabeled alanine as the plasma traversed the gut. To determine whether such exchange also took place across the liver the specific activity of alanine in plasma entering the liver was compared to that in plasma leaving the liver (Table II). The ratio of the two specific activities did not differ significantly from unity, thus indicating that little if any exchange took place as the plasma traversed the liver.

[1] *Abbreviations used in this paper*: Ra, rate of appearance; Rd, rate of disappearance.

TABLE I
*Control Period Plasma Alanine and Lactate Values**

	Alanine (n = 10)	Lactate (n = 10)
Arterial concentration, *μmol/liter*	250±23	589±80
Portal vein concentration, *μmol/liter*	291±22	680±76
Hepatic vein concentration, *μmol/liter*	143±11	350±82
Arterial sp act, *10^3 dpm/mol*	34.5±2.1	4.4±0.2
Portal vein sp act, *10^3 dpm/μmol*	23.1±1.3	4.1±0.2
Hepatic vein sp act, *10^3 dpm/μmol*	25.4±1.7	6.1±0.9
Net splanchnic uptake, *μmol/kg per min*	2.4±0.2	5.8±1.1
Net hepatic uptake, *μmol/kg per min*	3.1±0.2	7.6±0.9
Net gut production, *μmol/kg per min*	0.7±0.2	1.8±0.7
Ra, *μmol/kg per min*	7.4±0.2	—
Rd, *μmol/kg per min*	7.5±0.1	—

* For each dog the four consecutive control period values were averaged. The values shown are the overall mean±SEM for both groups of experiments. The mean weight of the dogs was 21.2±0.6 kg and mean estimated hepatic plasma flow was 23±3 ml/kg per min.

TABLE II
[^{14}C]Alanine Specific Activity (10^3 dpm/μmol) in Plasma Entering and Exiting the Liver during the Control Period of Each Experiment‡*

Dog	Exiting	Entering	Exiting/Entering
204	15.0	18.7	0.80
221	28.6	27.6	1.04
304	29.7	30.5	0.98
318	25.1	26.3	0.95
325	22.2	24.3	0.92
1,203	33.4	34.3	0.97
112	20.4	20.2	1.01
121	26.1	27.1	0.96
218	23.0	23.8	0.97
311	30.0	25.8	1.16
Mean	25.5	25.8	0.98
SEM	1.7	1.5	0.03

* The inflowing specific activity was calculated with the following formula: $(AC_p \times 0.72) + (AC_a \times 0.28)/(AR_p \times 0.72) + (AR_a \times 0.28)$, where AC is alanine concentration (micromole per milliliter); AR is alanine radioactivity (disintegration per minute per milliliter); p is portal plasma; and a is arterial plasma.

‡ For each dog the four consecutive control period values were averaged.

It is interesting to note that the net amount of alanine added by the gut (0.7 μmol/kg per min) was equivalent to 23% of the net alanine taken up by the liver. In addition, there was a wide disparity between the net rate of alanine uptake by the liver (3.1±0.2 μmol/kg per min) and the total flux of alanine out of the plasma compartment as measured by isotope dilution (7.5±0.1 μmol/kg per min). The mean rate of [^{14}C]alanine infusion was 239 × 10^3 dpm/kg per min, whereas the mean rate of splanchnic uptake was 111 × 10^3 dpm/kg per min. Thus, only 46% of the infused [^{14}C]alanine was removed by the splanchnic bed, indicating that 54% of the alanine flux from the plasma was occurring at extrasplanchnic sites.

90 min after the infusion of [^{14}C]alanine had begun, the arterial lactate specific activity was 13% of the arterial alanine specific activity (Table I). There was a slight drop (8%) in the lactate specific activity across the gut and a significant increase (46%) across the liver. The net amount of lactate added by the gut (1.8 μmol/kg per min) was equivalent to 24% of net lactate taken up by the liver (7.5 μmol/kg per min).

Fig. 1 shows the plasma insulin, glucagon, and glucose concentrations for the two groups. Infusion of glucose caused the plasma glucose level to rise by 115 mg/dl but did not elicit a change in the plasma level of either pancreatic hormone since the endocrine pancreas was inhibited by somatostatin and the hormones were being supplied by infusion. Net hepatic glucose

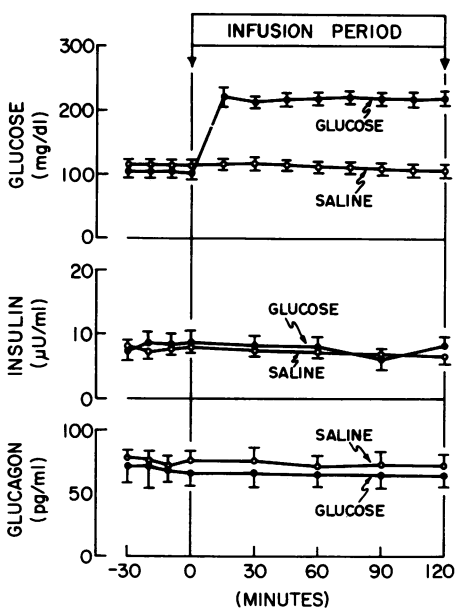

FIGURE 1 The arterial plasma glucose, insulin, and glucagon levels during saline or glucose infusions in 36-h-fasted conscious dogs maintained on infusions of somatostatin (0.8 μg/kg per min) and concurrent intraportal infusions of insulin (286 μU/kg per min) and glucagon (1 ng/kg per min).

TABLE III

*Hepatic Glucose Production during Saline or Glucose Infusion in Presence of Fixed Basal Insulin and Glucagon Levels**

	Control period				Test period							
min	−30	−20	−10	0‡	15	30	45	60	75	90	105	120
Saline	2.6	2.3	2.4	2.4	2.3	2.2	2.3	2.2	2.1	2.1	2.2	2.0
±SEM	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.4	0.2	0.1	0.1	0.2
Hyperglycemia	2.2	2.5	2.2	2.2	1.1	0.8	1.2	0.9	1.0	0.8	0.8	0.9
±SEM	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.3	0.3	0.2	0.2	0.2

* See Fig. 1.

‡ At zero time, a saline infusion was begun in one protocol while hyperglycemia was induced by exogenous glucose infusion in the other. The mean glucose infusion rate necessary to maintain the plasma glucose level at ~215 mg/dl was 3.34±0.1 mg/kg per min over the last 90 min of the study.

output (Table III) fell by ~60% in response to the hyperglycemia as previously reported (19).

The effect of hyperglycemia per se on alanine metabolism can be seen in Fig. 2. After the acute induction of hyperglycemia there was a progressive increase in the arterial plasma alanine concentration that continued unabated throughout the period of observation until it reached a level of 368±31 μmol/liter. This concentration was significantly different ($P < 0.01$) from both the mean control period value (238±18 μmol/liter) and the final concentration in the control group (210±18 μmol/liter). The rise in alanine was associated with a significant ($P < 0.01$ after 120 min) fall in the specific activity of the amino acid but little change in its radioactive concentration.

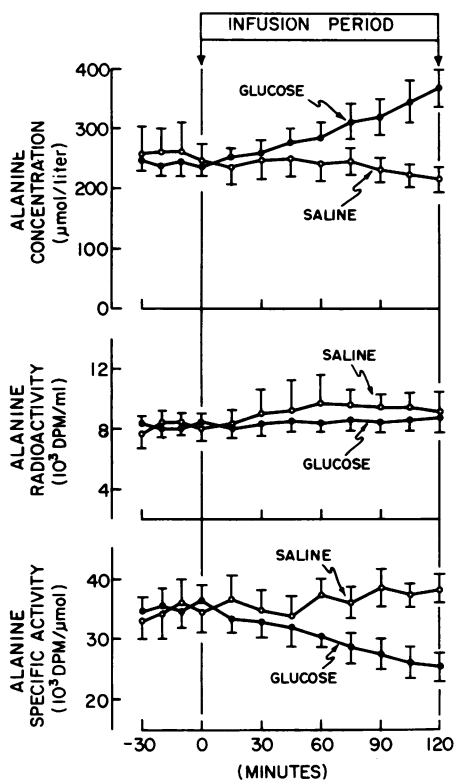

FIGURE 2 The effects of saline or glucose infusion on the concentration, radioactivity and specific activity of arterial plasma alanine in 36-h-fasted conscious dogs maintained on infusions of somatostatin (0.8 μg/kg per min) and concurrent intraportal infusions of insulin (286 μU/kg per min) and glucagon (1 ng/kg per min). The changes in the concentration and specific activity of alanine were significant ($P < 0.05$) from 90 min on when calculated using an unpaired t test and from 60 min on when calculated using a paired t test.

Table IV shows that net alanine uptake by the liver

TABLE IV

*Alanine Uptake** *before and during Saline or Glucose Infusion‡ in the presence of fixed Basal Insulin and Glucagon Levels§*

	Glucose		Saline	
Time	Net hepatic	Net splanchnic	Net hepatic	Net splanchnic
min				
−30	3.0±0.4	2.3±0.3	3.4±0.2	2.7±0.4
−20	3.2±0.4	2.2±0.3	3.2±0.1	2.5±0.2
−10	3.2±0.4	2.4±0.4	3.4±0.2	2.7±0.4
0	3.1±0.4	2.0±0.4	3.1±0.3	2.4±0.3
Infusion period				
15	3.0±0.3	2.2±0.3	3.3±0.3	2.3±0.2
30	3.2±0.3	2.1±0.2	3.0±0.4	2.4±0.3
45	3.2±0.3	2.3±0.3	3.1±0.4	2.7±0.4
60	3.0±0.3	2.1±0.3	3.4±0.5	2.5±0.4
75	3.2±0.2	2.3±0.4	2.7±0.4	2.2±0.4
90	3.3±0.3	2.1±0.4	2.5±0.4	2.1±0.3
105	3.2±0.3	2.2±0.4	3.0±0.4	2.3±0.2
120	3.5±0.2	2.5±0.3	3.5±0.4	2.3±0.2

Values are mean±SEM ($n = 5$).

* Alanine uptake (micromole per kilogram per minute).

‡ Saline or glucose were infused from 0 to 120 min.

§ See Fig. 1.

or splanchnic bed was not altered by hyperglycemia. Since the alanine level rose during the period of glucose infusion, however, the fractional extraction of the amino acid declined, and after 2 h had fallen by almost 20% ($P < 0.05$).

The effect of glucose infusion on the rates of appearance and disappearance of alanine from plasma are shown in Table V. In animals that received saline both Ra and Rd fell slightly during the course of the experiment. In contrast, the acute induction of hyperglycemia caused an immediate and progressive increase in both the Ra and Rd of alanine. Although Rd increased during hyperglycemia Ra always exceeded Rd (mean difference 0.19±0.06 μmol/kg per min) resulting in a net accumulation of alanine in the plasma compartment.

Fig. 3 depicts the effect of hyperglycemia per se on the arterial concentration and specific activity of lactate, as well as on net hepatic lactate uptake. The rise in the plasma lactate concentration induced by hyperglycemia was significant ($P < 0.05$) from 60 min on, whether compared to the mean basal value or to the lactate level in control group. There was a slight difference in the lactate specific activities in the two groups but it was only significant during the last 15 min of the study. Fig. 3 also shows that hyperglycemia caused the hepatic extraction of lactate to fall to a level not significantly different from zero after 60–90 min. As a consequence of continued lactate production by the gut, the splanchnic bed as a whole actually produced lactate during the last 30 min of the study (Table VI).

TABLE V

Ra and Rd of Plasma Alanine during Saline or Glucose Infusion in the Presence of Fixed Basal Insulin and Glucagon Levels‡*

	Glucose		Saline	
Time interval	Ra	Rd	Ra	Rd
min		*μmol/kg/min*		
−30 to −20	7.4±0.9	7.5±1.0	7.0±1.0	7.2±1.2
−20 to −10	7.6±0.7	7.5±1.1	6.8±1.2	6.8±1.1
−10 to 0	7.2±0.9	7.3±1.0	6.7±0.8	7.0±1.0
Infusion period				
0–15	7.8±1.0	7.6±0.9	6.5±0.8	6.5±0.8
15–30	7.9±0.9	7.9±0.9	6.7±1.0	6.5±1.1
30–45	8.2±1.0	8.0±1.0	6.6±0.9	6.6±0.9
45–60	8.5±1.0	8.4±1.0	6.0±0.8	6.1±0.8
60–75	9.0±1.0	8.8±1.0	6.0±0.8	6.1±0.8
75–90	9.4±1.0	9.3±1.1	5.8±0.7	5.9±0.7
90–105	9.9±1.0	9.7±1.0	5.7±0.7	5.6±0.8
105–120	10.0±1.0	9.8±1.7	5.5±0.6	5.7±0.6

The increases in Ra and Rd attributable to hyperglycemia were significant ($P < 0.05$) during the last hour of the experiment. Values are means±SEM ($n = 5$).

* Saline or glucose were infused from 0 to 120 min.

‡ See Fig. 1.

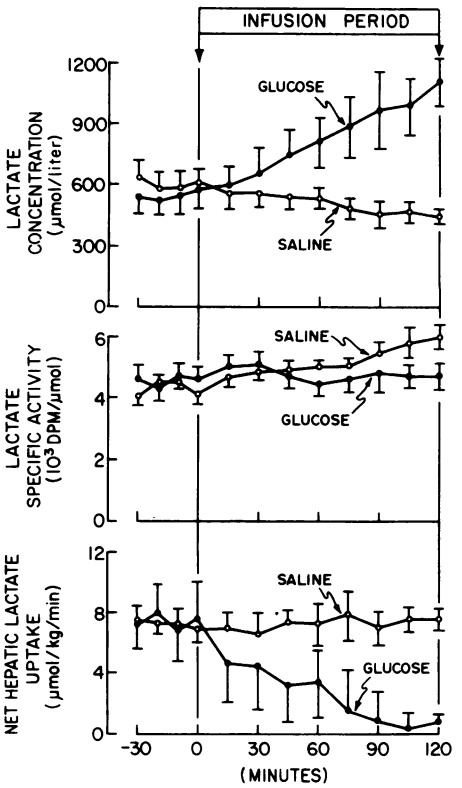

FIGURE 3 The effects of saline or glucose infusion on the concentration, specific activity and net hepatic uptake of plasma lactate in 36-h-fasted conscious dogs maintained on infusions of somatostatin (0.8 μg/kg per min) and concurrent intraportal infusions of insulin (286 μU/kg per min) and glucagon (1 ng/kg per min). The changes in the concentration and net hepatic uptake of lactate were significant ($P < 0.05$) from 90 min on when calculated using an unpaired t test and from 60 min on when calculated using a paired t test.

The data relating to [^{14}C]glucose are shown in Fig. 4. The specific activity of [^{14}C]glucose was 6% of the specific activity of alanine during the control period. Its value was not stable at that time, however, and continued to rise throughout the course of the control experiments. Infusion of unlabeled glucose to achieve hyperglycemia caused both the specific activity and production rate of [^{14}C]glucose to decrease markedly. In addition, the rate of conversion of circulating alanine and lactate to glucose was suppressed to <40% of its mean basal rate by the elevated blood glucose concentration.

Concentrations of individual amino acids in arterial plasma were determined by column chromatography in four out of five dogs that received glucose infusions. Table VII shows that hyperglycemia failed to induce any alteration in the arterial plasma concentrations of the branched chain amino acids. The plasma concentrations of all of the other amino acids, with the ex-

TABLE VI

*Splanchnic and Gut Lactate Balance during Saline or Glucose Infusion in Presence of Fixed Basal Insulin and Glucagon Levels**

	Control period				Test period							
min	−30	−20	−10	0*	15	30	45	60	75	90	105	120
					μmol/kg/min							
Splanchnic lactate uptake												
Saline	6.8	5.7	5.0	5.7	5.1	5.2	5.5	4.7	5.9	5.1	6.2	5.6
±SEM	1.5	1.2	1.2	0.9	0.8	1.3	1.2	1.5	1.5	0.6	0.9	0.5
Hyperglycemia	5.4	5.8	5.0	5.1	2.6	1.5	1.4	1.1	−1.2	−2.5	−2.7	−2.0
±SEM	1.0	1.2	0.7	0.7	1.8	2.7	2.2	2.5	2.3	1.2	0.7	0.3
Gut lactate production												
Saline	1.6	0.8	2.1	1.2	2.0	1.4	1.9	1.3	2.0	1.3	1.4	2.0
±SEM	0.7	0.8	0.4	0.5	0.5	0.8	1.1	0.9	0.8	0.2	0.5	0.3
Hyperglycemia	2.1	2.1	2.4	2.6	2.2	3.1	2.4	2.6	2.7	3.3	2.7	2.8
±SEM	0.7	0.8	1.2	0.8	1.1	0.6	1.1	0.8	1.1	1.0	0.7	1.6

* See Fig. 1.
‡ See Fig. 3.

ception of alanine, also failed to change significantly throughout the course of the experiments (data not shown).

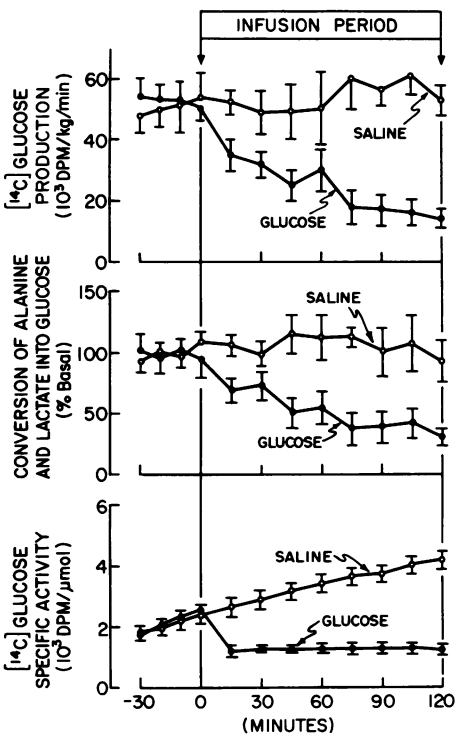

FIGURE 4 The effects of saline or glucose infusion on [^{14}C]-glucose production, the conversion of alanine and lactate into glucose and the plasma [^{14}C]glucose specific activity in 36-h-fasted conscious dogs maintained on infusions of somatostatin (0.8 μg/kg per min) and concurrent intraportal infusions of insulin (286 μU/kg per min) and glucagon (1 ng/kg per min).

DISCUSSION

The combination of arteriovenous difference and isotopic techniques used in these studies provided information that could not be gained by either technique alone. Catheterization of the artery as well as the portal and hepatic veins not only provided information concerning fluxes within the splanchnic bed, but also by isolating the splanchnic bed, allowed differentiation between the splanchnic and extrasplanchnic effects of hyperglycemia.

Basal splanchnic alanine exchange. The control period data indicate that the dilution of the alanine specific activity that has been reported to occur across the splanchnic bed in man (22) and the dog (23) takes place in the extrahepatic tissues drained by the portal vein. Inasmuch as little if any dilution occurs within the liver, the flux of alanine must be inward in this

TABLE VII

Arterial Plasma Branched Chain Amino Acid Concentrations before and during Glucose Infusion in the Presence of Fixed Basal Insulin and Glucagon Levels‡*

Time	Leucine	Isoleucine	Valine
min		*μmol/liter*	
−30	116±15	75±3	168±16
0	117±15	76±1	166±9
30	108±10	75±3	165±14
60	113±10	85±5	170±18
90	107±12	77±5	166±16
120	109±11	72±2	150±8

Values are means±SEM (n = 4).
* Glucose was infused from 0 to 120 min.
‡ See Fig. 1.

organ, or bidirectional, but so rapid that the intracellular and plasma [^{14}C]alanine specific activities are equal.

Basal alanine turnover. Certain theoretical considerations of tracer methodology must be kept in mind when interpreting the alanine turnover data. The Ra of a substance in a given pool, or its equivalent of irreversable disposal, can be calculated under steady-state conditions using the plateau specific activity of the labeled moiety in the infused pool regardless of the configuration of the system or whether other sites of input and output exist (24). The Ra determined in this manner, however, is equivalent only to the rate of input of new trace to the pool sampled, and is not necessarily equivalent to the total turnover of the substances within the body, the latter being dependent upon the model assumed for the system as a whole (24). If production and usage are occurring in an intracellular compartment, the determination of turnover in the central compartment (plasma or extracellular space) will underestimate total turnover unless equilibrium between the extra and intracellular pools is so rapid that the specific activity is the same in both compartments (24).

During the control period of the present studies the Ra and Rd of alanine were equal and were, as indicated above, a measure of the flux of new alanine into and out of the plasma compartment. Net alanine uptake by the liver equalled net alanine production by extrahepatic tissues, at that time, since the plasma alanine concentration was stable. While it would not be expected that the total alanine flux and net entry of alanine into plasma would be identical, it is of interest that the rate of total alanine flux was over twice the rate of net alanine entry into plasma. Furthermore, >50% of total alanine efflux occurred in extrasplanchnic sites. As mentioned above, interpretation of these findings is dependent upon the model assumed for the alanine system. The current data cannot rule out net transport of alanine from muscle to other extrasplanchnic organs as an explanation for the large turnover rate of the amino acid, but data in the literature suggest that other tissues such as the brain (25) and kidney (26, 27) take up little if any alanine. Because of the observation in man that [^{14}C]alanine is extracted by forearm muscle even when there is net addition of alanine across that vascular bed (28), it seems more likely that the high turnover rate results from exchange of labeled and unlabeled molecules in muscle.

Hyperglycemia and alanine metabolism. The ability of hyperglycemia per se to increase net alanine entry into the circulation in the present study (as shown by the alanine level rising in the face of an unchanged hepatic extraction rate) is confirmed by the isotopically determined Ra and Rd of alanine. Because the initial event was an increase in Ra rather than a fall in Rd it is apparent that it was the increase in the Ra of alanine that triggered the rise in the alanine concentration. The Ra of alanine had increased by 33% by the end of the period of glucose infusion. Rd also increased throughout the hyperglycemic period but was always somewhat less than Ra. The average increment in the net rate of entry of alanine into the circulation (i.e., the difference between Ra and Rd) over the entire period was 2.5%. Since the alanine space may be larger than the space that we assumed (i.e., glucose space), the data were also calculated using a volume of distribution equal to total body water. In that case, Ra increased from a mean control value of 7.4 to 10.7 μmol/kg per min, an increment of 45%. Without knowledge of the exact alanine space one cannot be certain of the magnitude of increase in the rate of alanine production, but since the amino acid must distribute in a space at least equal to the glucose space we can conclude that its rate of appearances must have risen by a minimum of 33%.

As mentioned, the interpretations of these changes are dependent upon the compartment model assumed for alanine in vivo. If it is assumed, as would be suggested by the studies across human forearm (28), that much of the observed flux of alanine in the current studies was occurring in muscle then the data suggest that the increase in Ra was caused by an increase in intracellular alanine (pyruvate) turnover as well as increase in the net addition of alanine to the plasma. Because intracellular alanine is in rapid equilibrium with pyruvate it is possible that the increased alanine turnover actually reflects increased pyruvate turnover in the cell.

This in vivo demonstration that hyperglycemia is capable of increasing alanine production is in agreement with the recent in vitro studies of Chang and Goldberg (3) but at odds with the conclusion of Garber et al. (5) that alanine production in muscle in vitro is not influenced by glucose. Both of the above groups point out the source of alanine in muscle is twofold; de novo synthesis and proteolysis. Because it is unlikely that hyperglycemia would increase proteolysis, and indeed no changes in the concentration of amino acids other than alanine could be detected, it would seem that the de novo synthesis of alanine is influenced by the plasma glucose concentration. This finding thus offers an explanation for the failure of oral or intravenous glucose to suppress the plasma alanine concentration while markedly suppressing the levels of other amino acids (13, 14) in that increased *de novo* synthesis of alanine probably offsets decreased alanine released from protein brought about by the anabolic effects of combined hyperglycemia and hyperinsulinemia.

The failure of hyperglycemia to suppress net hepatic alanine uptake does not mean that it was totally without effect on alanine extraction by the liver. The rising concentration of alanine in the artery and portal vein

should have increased hepatic alanine extraction.[2] The fact that this did not happen suggests that the efficiency of alanine removal by the liver was impaired, as indicated by a fall in the fractional extraction of the amino acid (20%) as well as a fall (16%) in overall alanine clearance. Although this fall was not the cause of the increase in the arterial alanine concentration, a decrease in the efficiency of the liver to extract alanine undoubtedly contributed to the continued rise in the concentration of the amino acid.

Felig et al. (13, 14) have shown that both intravenous and oral glucose acutely suppress net splanchnic alanine extraction in man. We have previously shown in dogs that the infusion of insulin with euglycemia maintained was without effect on splanchnic alanine extraction (29). The present study also indicates that hyperglycemia in the presence of basal insulin and glucagon concentrations does not significantly suppress net hepatic alanine uptake. Taken as a whole these observations suggest that concomitant hyperglycemia and hyperinsulinemia are necessary to suppress net hepatic alanine extraction or that other factors, perhaps a decrease in the plasma glucagon level, are responsible for the observations of Felig et al. (13, 14).

Interchange of alanine and lactate. Kreisberg et al. (30) and more recently Chochinov et al. (11, 12) have observed a rapid appearance of ^{14}C in lactate after the infusion of [^{14}C]alanine. They point out that such could occur indirectly via conversion of [^{14}C]glucose to [^{14}C]-lactate in the periphery or directly via [^{14}C]pyruvate in the liver. The control period lactate data indicate that considerable direct conversion of [^{14}C]alanine to [^{14}C]lactate occurs within the liver since the lactate specific activity in plasma increased by 46% across this organ. Such enrichment could have not occurred by conversion of [^{14}C]glucose to lactate since during this time the [^{14}C]glucose specific activity was less than half that of the lactate. Our finding that 13% of the lactate was derived from alanine is in close agreement with the finding of Chochinov et al. (11, 12) in man.

Hyperglycemia and lactate metabolism. After 2 h of hyperglycemia the plasma lactate concentrations had doubled, primarily as a result of an almost complete cessation of lactate uptake by the liver. Whole blood lactate levels were not routinely determined, but in one experiment both plasma and whole blood were assayed for lactate and the changes in whole blood lactate were proportional to the changes in plasma lactate although basal lactate uptake by the liver was 27% higher when whole blood was used. The mechanism by which the change in net hepatic balance occurs is not known, but it is tempting to speculate that an elevated glucose concentration occurring in the absence of an appropriate insulin to glucagon molar ratio might lead to enhanced glycolysis and an increased level of lactate in the liver cell. Since the net movement of lactate into the liver is dependent upon its concentration gradient across the membrane the rise in intracellular lactate would impair its net uptake by hepatocytes. A similar inhibition of splanchnic lactate uptake was observed by Felig et al. (14) after glucose administration to man.

Hyperglycemia and the conversion of alanine and lactate to glucose. The method used for calculation of alanine and lactate conversion to glucose quantifies the rate of conversion of circulating arterial plasma alanine and lactate to plasma glucose. As previously discussed the method does not quantify total gluconeogenesis, nor can it identify a mechanism by which a change in conversion is brought about (18). The data nevertheless indicate that the rate of conversion was reduced by 60% after the induction of hyperglycemia. This decrease could not be accounted for by deposition of [^{14}C]glucose in glycogen since analysis of liver biopsies at the end of each experiment revealed that the glycogen present (18 mg/g tissue) was unlabeled. The current data indicate that part of the hyperglycemic effect on gluconeogenic conversion can be accounted for by the suppression of net hepatic lactate extraction. Only 20% of the ^{14}C counts extracted by the liver during the control period were in lactate, however, so that complete suppression of the uptake of this precursor could only account for a 20% reduction in conversion. Because suppression of precursor conversion to glucose was much >20%, and because alanine uptake in absolute terms did not change, an additional intrahepatic effect of hyperglycemia is suggested. This could come about through an inhibition of the gluconeogenic pathway per se, or an enhancement of glycolysis that would also involve a dilution of the intracellular gluconeogenic precursor specific activity. Whether the cause of the decreased appearance of [^{14}C]alanine in [^{14}C]-glucose is direct inhibition of gluconeogenesis or stimulation of glycolysis it must reflect a decrease in the net flux of gluconeogenic carbon to glucose within the liver. Evidence is available from in vitro experiments to support the contention that glucose per se can inhibit gluconeogenesis (31–33).

When interpreting the physiological significances of the current findings it should be borne in mind that the glucagon and insulin concentrations were kept constant and not allowed to vary appropriately in response to hyperglycemia. Whether the usual changes in these two hormones that normally accompany hyperglycemia would potentiate or inhibit the observed changes in alanine and lactate flux is not known. The data reported by O'Connell et al. (34) and Fitzpatrick et al. (35) that showed that glucose administration at high rates (intravenously or orally) increases the plasma alanine con-

[2] Rollings, R., M. Diamond, P. Williams, A. D. Cherington, and W. W. Lacy. Unpublished observation.

centration would suggest that the effects of hyperglycemia are not offset by concurrent changes in insulin or glucagon. Although Waterhouse and Kleison (36) were unable to detect an effect of glucose infusion on alanine metabolism in malnourished female subjects the rate of infusion was small and resulted in a glucose level of only about 125 mg/dl. In the one of their patients who received twice as much glucose the effect on alanine metabolism was entirely consistent with our observations.

The current data do not contradict the evidence that glucagon and insulin play important roles in the control of alanine and lactate metabolism in vivo. They suggest, however, that the plasma glucose level can itself also modify the metabolism of the two main gluconeogenic precursors. Hyperglycemia leads to an elevation in the plasma lactate concentration primarily as a consequence of decreased lactate uptake by the liver. In addition, it causes the plasma alanine level to rise as a result of an increase in the production rate of the amino acid and a fall in its fractional extraction by the liver. Lastly, hyperglycemia not only alters the net rate of entry of the aforementioned gluconeogenic precursors into the liver, it also decreases the efficiency with which they are converted to glucose.

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