Effects of Amino Acids on Glucose Disposal

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Free fatty acids are known to inhibit carbohydrate disposal and oxidation. This action may play an important role in the pathophysiology of insulin resistance and non-insulin-dependent diabetes mellitus. To investigate whether amino acids (AAs) have similar actions, we determined the effects of an intravenously infused mixture of 15 AAs on carbohydrate disposal during euglycemic-hyperinsulinemic clamps associated with either basal or high glucagon concentrations in healthy male volunteers. Plasma glucose concentration was clamped at ~4.7 mM (coefficient of variation 4.7%). Insulin infusion (7.18 pmol · kg^{-1} · min^{-1}) raised serum insulin concentrations from 36–50 pM to between 300 and 600 pM. AA infusions (0.5 g · kg^{-1} · h^{-1} · 4 h) raised plasma α-amino N_2 concentrations about five- to six-fold. Infusion of AAs, somatostatin (somatotropin release inhibitory factor, SRIF), and high-glucagon replacement (3.0 ng · kg^{-1} · min^{-1}) reduced the rate of exogenous glucose infusion needed to maintain euglycemia from 51.1 ± 7.2 μmol · kg^{-1} · min^{-1} (saline + SRIF + high glucagon) to 28.3 ± 11.1 μmol · kg^{-1} · min^{-1} and stimulated endogenous glucose production (from 0 to ~17 μmol · kg^{-1} · min^{-1}). Thus, glucose disposal (exogenous infusion plus endogenous production of glucose) remained essentially unchanged. During infusion of AAs + SRIF + basal glucagon replacement (0.25 ng · kg^{-1} · min^{-1}), endogenous glucose production remained completely suppressed, and the rates of exogenous glucose infusion did not change (compared with saline + SRIF + basal glucagon replacement). The data showed that *1*) hyperaminoacidemia associated with hyperglucagonemia stimulated endogenous glucose production despite hyperinsulinemia, and *2*) intravenous infusion of a mixture of 15 AAs had no inhibitory effect on insulin-stimulated total-body glucose disposal. *Diabetes* 39:1079–84, 1990

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Free fatty acids (FFAs) have long been known to interfere with carbohydrate utilization (1,2). Competition between FFA and carbohydrate as fuels for oxidation has been suggested to play an important role in the pathophysiology of insulin resistance and non-insulin-dependent diabetes (NIDDM; 1–6). However, whether amino acids (AAs) have similar effects on carbohydrate utilization remains uncertain. Some AAs, particularly leucine, isoleucine, and their ketoacid analogues, inhibit glucose oxidation in vitro (7,8), and intravenous infusion of leucine, isoleucine, threonine, and α-ketoisocaproate decrease human forearm glucose uptake (9,10). However, the effects of a mixture of several AAs were tested in only one study and found to decrease the rate of glucose infusion needed to maintain euglycemia during hyperinsulinemic clamps by 36% in healthy subjects (11). Hepatic glucose output was not measured in this study, and thus it could not be determined whether the infused AAs actually changed glucose disposal. Therefore, we restudied the effect of an intravenously infused mixture of 15 AAs on glucose disposal by measuring rates of endogenous glucose production and exogenous glucose infusion needed to maintain euglycemia during hyperinsulinemic clamps in healthy male volunteers.

RESEARCH DESIGN AND METHODS

Twenty-eight studies were performed with 17 healthy male subjects with three protocols. Six subjects (aged 25.2 ± 1.6 yr [range 21–32 yr], weight 83.1 ± 5 kg [range 73–101 kg]) were studied in protocol 1. Five subjects (aged 23.8 ± 0.8 yr [range 21–25 yr], weight 79.6 ± 6.0 kg [range 71–89 kg]) were studied in protocol 2. Six subjects (aged 23.2 ± 1.8 yr [range 19–28 yr], weight 78.3 ± 4.0 kg [range 66.4–90.0 kg]) were studied in protocol 3. None of the subjects were obese. The heaviest individual (101 kg, 190.5 cm) was a

nonobese world-class athlete with a large muscle mass. None of the subjects had a family history of diabetes or other endocrine disorders, and none were taking any medications. Their weight-maintaining diets contained a minimum of 200 g/day carbohydrate. Informed written consent was obtained from all subjects after an explanation of the nature, purpose, and potential risks of each study. The study protocol was approved by the institutional review board for human research at Temple University. Studies were performed in the General Clinical Research Center of Temple University and began between 0800 and 0900 after an overnight fast. The subjects were studied reclining in beds.

Protocol 1. Six subjects were studied twice, in random order, once with AAs and once with saline. A minimum of 3 wk was allowed between studies. Regular human insulin (Humulin R, Lilly, Indianapolis, IN) was infused (7.18 pmol · kg^{-1} · min^{-1} i.v.) for 6 h. Glucose concentrations were clamped at ~4.7 mM by a feedback-controlled glucose infusion (Biostator, Ames, Elkhart, IN). Blood for the Biostator was continuously drawn from a superficial antecubital vein. Two hours were allowed for attainment of steady-state hyperinsulinemia. At 120 min, AAs (0.5 g · kg^{-1} · h^{-1}) or saline was infused intravenously. With the exception of acetate, which was omitted, our AA solution was identical to 7% aminosyn (Abbott, North Chicago, IL), which is used for parenteral nutrition. This solution contains relatively high concentrations of branched-chain AAs (leucine, isoleucine, valine), which are known to rise prominently after a high-protein meal (12). The AA solution contained the following L-AAs: isoleucine (5.1 g/L), leucine (6.6 g/L), lysine (5.1 g/L), methionine (2.8 g/L), phenylalanine (3.1 g/L), threonine (3.7 g/L), tryptophan (1.2 g/L), valine (5.6 g/L), alanine (9.0 g/L), arginine (6.9 g/L), histidine (2.1 g/L), proline (6.1 g/L), serine (3.0 g/L), tyrosine (0.4 g/L), and glycine (9.0 g/L). These AAs, purchased from Sigma (St. Louis, MO), were dissolved in distilled water. The solutions were filtered through 0.02-mm Milipore filters, autoclaved, and tested for pyrogenicity (limulus amoebocyte lysate test, Marine Biologicals, Marmora, NJ) before use. [3-^{3}H]glucose (Du Pont-NEN, Boston, MA) was infused for 6 h starting with a 40-μCi bolus followed by a continuous infusion of 0.4 μCi/min. Venous blood samples for determination of substrates and hormones were obtained at 30-min intervals between 0 and 360 min.

Protocol 2. Subjects were studied twice, in random order, with infusions of AAs, somatostatin (somatotropin release inhibitory factor, SRIF), and glucagon (AA + SRIF + glucagon 3.0 ng · kg^{-1} · min^{-1}, n = 5) and with infusions of saline, SRIF, and glucagon (saline + SRIF + glucagon 3.0 ng · kg^{-1} · min^{-1}, n = 3). Infusions of insulin, glucose, [3-^{3}H]glucose, and AAs (0.5 g · kg^{-1} · h^{-1}) or saline and collections of blood were as described in protocol 1. In addition, cyclic SRIF (306 nmol/h) was infused, starting at 115 min to suppress endogenous release of insulin and glucagon. Glucagon was infused starting at 115 min at a dose (3.0 ng · kg^{-1} · min^{-1}) estimated to simulate portal venous glucagon concentrations after AA stimulation.

Protocol 3. Infusions were as described in protocol 2, except that glucagon was infused at a rate of 0.25 ng · kg^{-1} · min^{-1}. This dose was chosen to replace basal glucagon concentrations. Five subjects received infusions of AAs, SRIF, and glucagon (AA + SRIF + glucagon 0.25), and three subjects received infusions of saline, SRIF, and glucagon (saline + SRIF + glucagon 0.25).

Hepatic glucose output was calculated by subtracting the rate of glucose infusion needed to maintain euglycemia from the isotopically determined rate of glucose appearance (R_a). R_a was determined with [3-^{3}H]glucose dilution analysis. Two hours were allowed for equilibration of [3-^{3}H]glucose. Plasma glucose was isolated from blood drawn at 60-min intervals for determination of [3-^{3}H]glucose specific activity as described (13). R_a were calculated for 60-min intervals with the equations of Steele (14) for non-steady-state conditions. R_a during saline infusions were frequently lower than the respective glucose infusion rates, resulting in negative values for hepatic glucose production. This problem has recently been reported to be caused at least partially by a nonglucose ^{3}H-labeled contaminant of the [^{3}H]glucose trace (15). Thus, when the isotopically determined R_a was equal or smaller than the rate of exogenously infused glucose, it was assumed that endogenous glucose production was completely suppressed.

Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA). Serum insulin (16) and plasma glucagon (17) concentrations were determined by radioimmunoassay with Unger's COOH-terminal–specific 30K antiserum (17). Plasma α-amino N_2 concentrations were measured colorimetrically (18). Plasma FFAs were measured according to Lorch and Gey (19) after extraction according to Dole and Meinertz (20). All data are means ± SE. Statistical significance was assessed using multiple analyses of variance or Student's paired or unpaired t test.

RESULTS

Substrates and hormones. Plasma glucose concentrations were clamped at ~4.7 mM (coefficient of variation 4.7%) during all studies (Table 1). During AA infusions, plasma α-amino N_2 concentrations increased 5- to 6-fold ($P < 0.001$) from 0.26 ± 0.04 (at 120 min) to 1.54 ± 0.2 mmol/dl (AA at 360 min), from 0.33 ± 0.06 to 1.68 ± 0.28 mmol/dl (AA + SRIF + glucagon 0.25), and from 0.32 ± 0.03 to 1.52 ± 0.14 mmol/dl (AA + SRIF + glucagon 3.0).

Insulin infusion (7.18 pmol · kg^{-1} · min^{-1}) raised serum insulin concentrations ~10-fold from between 36 and 50 pM to plateau concentrations between 300 and 600 pM in all protocols except during AA infusions when insulin concentrations increased further to a maximum of 976 ± 538 pM. This additional increase was seen in only three of six subjects.

Plasma glucagon remained at basal concentrations (<100 ng/L) during infusions of saline or AAs + SRIF + basal glucagon. During infusion of AAs alone, glucagon rose continuously to a maximum of 316 ± 47 ng/L. During infusions of saline + SRIF + high glucagon or AAs + SRIF + high glucagon, it rose to maxima of 498 ± 52 and 420 ± 14 ng/L, respectively. Plasma FFA concentrations decreased to <100 μM during insulin infusions in all studies.

Exogenous glucose infusion and endogenous glucose production rates. During saline infusions, when hyperinsulinemia was associated with basal glucagon concentrations, glucose infusion rates rose from 0 at baseline to 51.1 ± 7.2 μmol · kg^{-1} · min^{-1} (at 360 min) (Fig. 1, *upper panel*). During AA infusions, when hyperinsulinemia was as-

TABLE 1
Substrates and hormones

Studies	Time (min)				
	120	180	240	300	360
Glucose (mM)					
AA	4.82 ± 0.11	4.87 ± 0.17	4.76 ± 0.22	5.04 ± 0.39	5.15 ± 0.39
+ Basal glucagon	4.59 ± 0.11	4.82 ± 0.11	4.82 ± 0.11	4.65 ± 0.06	4.70 ± 0.06
+ High glucagon	4.87 ± 0.06	4.93 ± 0.11	4.82 ± 0.06	4.87 ± 0.06	4.87 ± 0.06
Saline	4.70 ± 0.06	4.76 ± 0.11	4.76 ± 0.22	5.04 ± 0.39	5.15 ± 0.39
+ Basal glucagon	4.76 ± 0.11	4.98 ± 0.06	5.26 ± 0.34	4.93 ± 0.17	4.76 ± 0.34
+ High glucagon	4.20 ± 0.45	4.59 ± 0.34	4.54 ± 0.45	4.87 ± 0.84	4.26 ± 0.67
α-NH_2 N_2 (mmol/dl)					
AA	0.26 ± 0.04	1.02 ± 0.05	1.26 ± 0.16	1.40 ± 0.18	1.54 ± 0.20
+ Basal glucagon	0.33 ± 0.06	1.00 ± 0.09	1.46 ± 0.14	1.65 ± 0.25	1.68 ± 0.28
+ High glucagon	0.32 ± 0.03	1.12 ± 0.08	1.28 ± 0.09	1.35 ± 0.06	1.52 ± 0.14
Saline	0.26 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.22 ± 0.01
+ Basal glucagon	0.26 ± 0.04	0.22 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.17 ± 0.02
+ High glucagon	0.31 ± 0.03	0.30 ± 0.02	0.43 ± 0.11	0.30 ± 0.01	0.32 ± 0.04
Insulin (pM)					
AA	345 ± 57	905 ± 316	625 ± 215	854 ± 323	976 ± 538
+ Basal glucagon	503 ± 50	546 ± 29	538 ± 43	567 ± 29	431 ± 65
+ High glucagon	323 ± 36	409 ± 29	373 ± 36	352 ± 29	517 ± 93
Saline	330 ± 43	431 ± 57	359 ± 29	366 ± 50	416 ± 29
+ Basal glucagon	481 ± 50	402 ± 79	380 ± 72	395 ± 57	359 ± 86
+ High glucagon	460 ± 14	452 ± 14	380 ± 29	388 ± 43	352 ± 36
Glucagon (ng/L)					
AA	58 ± 6	189 ± 4	267 ± 62	265 ± 48	316 ± 47
+ Basal glucagon	78 ± 4	79 ± 6	78 ± 8	75 ± 6	106 ± 18
+ High glucagon	71 ± 26	368 ± 55	381 ± 54	449 ± 130	498 ± 52
Saline	57 ± 18	44 ± 7	38 ± 6	42 ± 4	35 ± 6
+ Basal glucagon	67 ± 8	71 ± 5	69 ± 9	75 ± 2	74 ± 5
+ High glucagon	166 ± 81	420 ± 14	343 ± 54	376 ± 23	345 ± 42

Values are means ± SE. n = 6 for amino acid (AA) and saline. n = 5 for AA + basal (0.25 ng · kg^{-1} · min^{-1}) glucagon and AA + high (3 ng · kg^{-1} · min^{-1}) glucagon. n = 3 for saline + basal glucagon and saline + high glucagon.

sociated with high glucagon concentrations, glucose infusion rates rose from 0 to 28.3 ± 11 μmol · kg^{-1} · min^{-1}. This 45% decrease in the rate of exogenous glucose infusion ($P < 0.02$) was explained by an increase in the rate of endogenous glucose production. During saline infusions, endogenous glucose production, although not measured in those experiments, is completely suppressed by the rate of insulin infused in these studies (21). During AA infusions, endogenous glucose production rose continuously from 0 μmol · kg^{-1} · min^{-1} at 120 min to 16.1 ± 1.7 μmol · kg^{-1} · min^{-1} at 360 min ($P < 0.002$). Because the sum of exogenous glucose infusions plus endogenous glucose production was not significantly different during saline and AA infusions, glucose disposal did not appear to be inhibited by AAs.

AAs or saline + SRIF + high glucagon. Because insulin concentrations were higher during AA compared with saline infusions in three of six subjects, we performed a second series of experiments in which the rise of insulin in response to AAs was prevented by somatostatin, and glucagon was infused in doses to simulate portal venous glucagon concentrations seen during AA infusions (22; Fig. 1, *middle panel*). The results were similar to those seen with infusion of AAs or saline alone. At the end of the study, glucose infusion rates were 48.9 ± 12.8 μmol · kg^{-1} · min^{-1} with saline and 28.3 ± 4.4 μmol · kg^{-1} · min^{-1} with AAs (−42%, $P < 0.05$). Endogenous glucose production remained completely suppressed during saline but rose to 15.6 ± 2.8 μmol · kg^{-1} · min^{-1} at 360 min ($P < 0.01$) during AA infusions. The data from both studies suggested that hyperglucagonemia (300–500 ng/L) was able to increase endogenous glucose production despite insulin suppression. This, in turn, reduced the need for exogenous glucose infusion to maintain euglycemia.

AAs or saline + SRIF + basal glucagon. To further test this hypothesis, we performed a third series of experiments identical to the second except that glucagon was infused at a dose to produce basal glucagon concentrations (Fig. 1, *lower panel*). During those studies, endogenous glucose production remained suppressed, and exogenous glucose infusion and thus glucose disposal were the same during saline and AA infusions.

DISCUSSION

The main conclusion derived from these studies was that infusion of a large AA load (2 g · kg^{-1} · 4 h^{-1}) did not interfere with insulin-stimulated total-body glucose disposal. This conclusion was supported by data obtained under three different experimental conditions. In protocols 1 and 2, conditions were comparable to those seen after a large protein meal (19 oz of roasted turkey meat; 23); i.e., hyperaminoacidemia was associated with euglycemia, hyperinsulinemia, and hyperglucagonemia. The results obtained in both protocols indicated that AA infusions increased endogenous glucose production but did not interfere with glucose disposal. These results depended to some extent on the accuracy of the [3-^{3}H]glucose–turnover data. After completion of our studies,

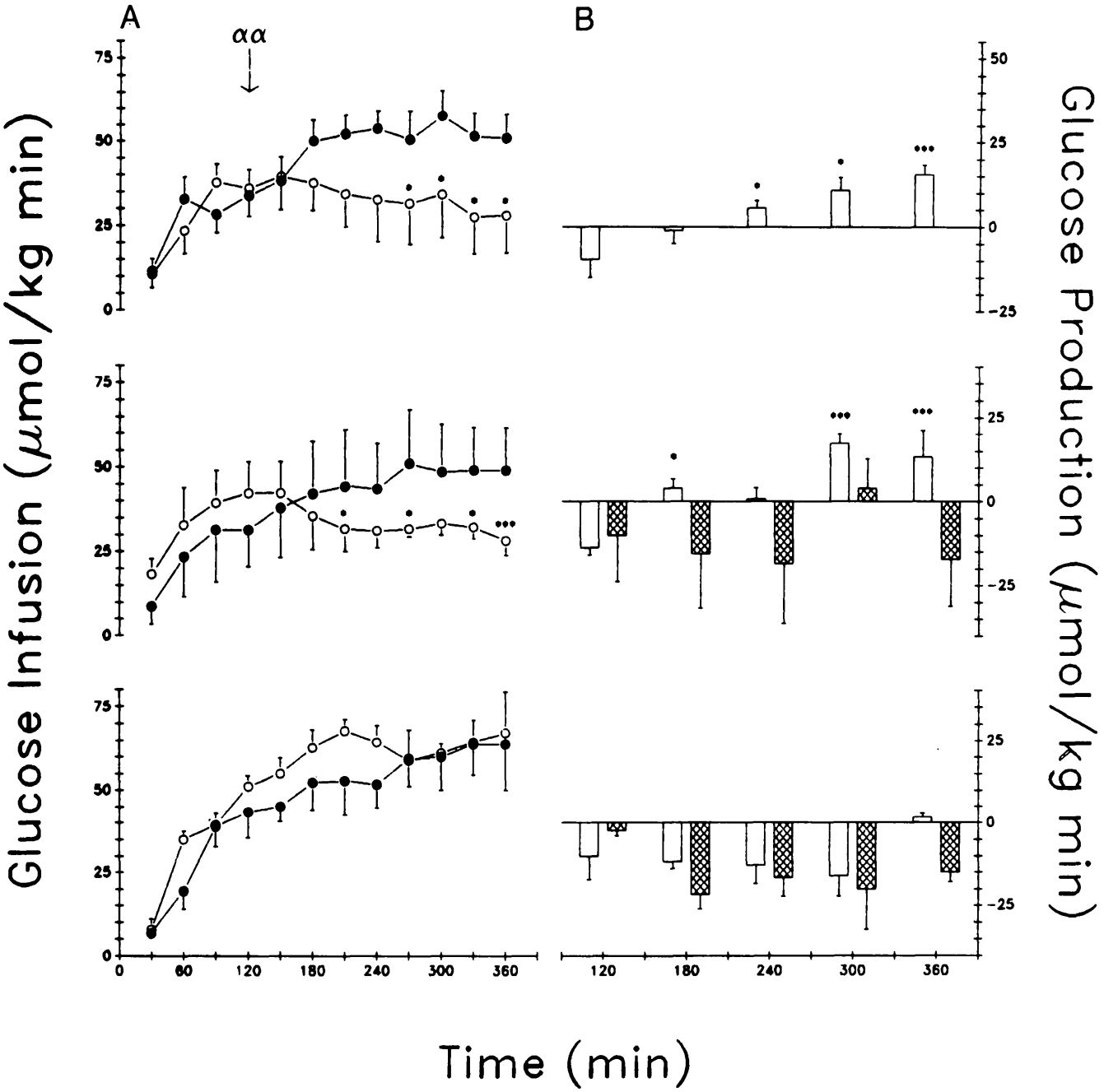

FIG. 1. *A*: *upper panel*, glucose infusion rates needed to maintain euglycemia during insulin (7.18 pmol · kg^{-1} · min^{-1}), amino acid (AA, ○; 2.0 g · kg^{-1} · 4 h^{-1}; *n* = 6), or saline (●; *n* = 6) infusions. Values are means ± SE. Analysis of variance for repeated measures was used to evaluate overall difference between saline and AA infusions. Student's *t* test was used to determine at what time 2 infusions were significantly different. *Middle panel*, infusions of insulin, AA (*n* = 5), or saline (*n* = 3) were same as in *upper panel*. In addition, somatostatin (306 nmol/h) was infused to prevent endogenous insulin release in response to AAs, and glucagon was replaced at rate of 3.0 ng · kg^{-1} · min^{-1}. *Lower panel*, infusions and symbols as in *middle panel* except that glucagon was replaced at rate of 0.25 ng · kg^{-1} · min^{-1} (*n* = 5 for AA infusions, *n* = 3 for saline infusions). *B*: glucose production rates (isotopically determined rate of glucose appearance minus glucose infusion rates) during same experiments as shown in *A*. *Open bars*, glucose production during AA infusions; *crosshatched bars*, glucose production during saline infusions. Values are means ± SE. *$P < 0.05$; ***$P < 0.005$.

a report showed that some commercially available batches of [^{3}H]glucose were contaminated with a ^{3}H-labeled non-glucose substance that coeluted with glucose from the ion-exchange column used to isolate [^{3}H]glucose and caused underestimation of glucose turnover (15). Negative glucose production rates observed during most saline infusions and at the beginning of the AA infusions indicated that our measured rates of glucose appearance were indeed too low. However, this potential error does not invalidate our conclusion, because greater endogenous glucose production rates further reduce the possibility that AAs inhibited glucose disposal. In addition, our observation that [^{3}H]glucose–specific activities remained essentially constant during the last 2–3 h (Fig. 2) while glucose infusion rates fell (AAs, AA plus high glucagon; Fig. 1) indicated that hepatic glucose production must have increased during these studies.

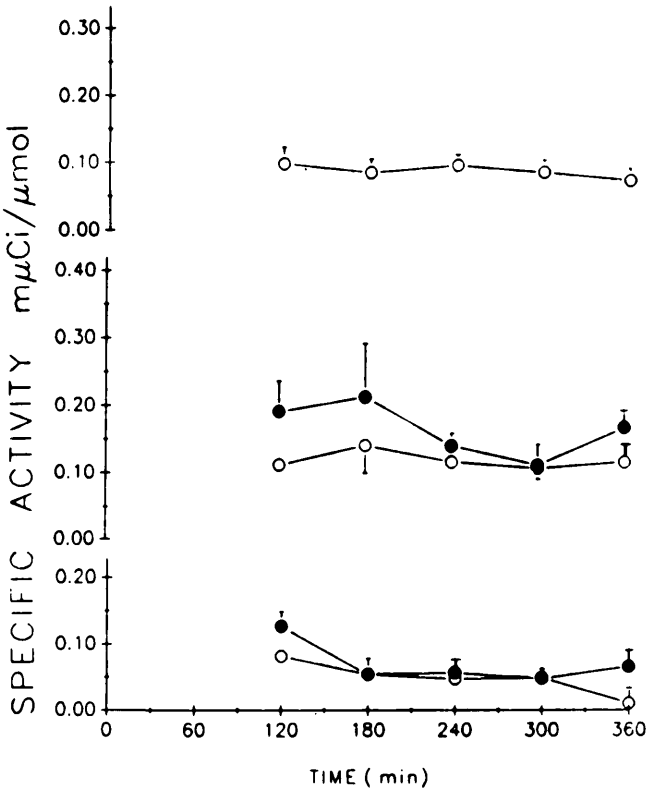

FIG. 2. [3-^{3}H]glucose-specific activities. *Upper panel*, infusion of amino acids (AAs; ○) alone; *middle panel*, infusion of AAs or saline (●) together with somatostatin and glucagon (3.0 ng · kg^{-1} · min^{-1}); *lower panel*, infusion of AAs and glucagon (0.25 ng · kg^{-1} · min^{-1}). Values are means ± SE.

In protocol 3 (hyperaminoacidemia associated with euglycemia, hyperinsulinemia, and basal glucagon concentrations), endogenous glucose production remained completely suppressed. Hence, the influence of falsely low glucose turnover rates on calculated glucose disposal rates was largely eliminated. (Suppression of endogenous glucose production under these conditions has also been demonstrated with [^{14}C]glucose, which does not have the problems associated with [^{3}H]glucose [15].) The results confirmed that glucose disposal rates, which in this case equaled rates of exogenous glucose infusions, were identical during AA and saline infusions.

The late infusion of AAs (2 h after glucose infusion) in our studies may have compromised the ability of AAs to compete with glucose for utilization. Although we cannot exclude this possibility, we consider it unlikely, because we have previously shown that ethanol, also infused 2 h after glucose infusion, inhibited glucose utilization (24).

Our findings agree with data published by Tessari et al. (11), who used an experimental protocol very similar to ours (a mixture of 14 AAs infused during euglycemic hyperinsulinemia) and observed a 36% reduction in the rate of glucose infusion. These authors concluded that AAs inhibited glucose disposal based on their assumption that hepatic glucose output, which was not measured, remained suppressed by hyperinsulinemia. This assumption was probably incorrect. Our studies showed that hyperaminoacidemia combined with hyperglucagonemia stimulated endogenous glucose production even in the presence of hyperinsulinemia (in the 350–1000-pM range). Neither hyperglucagonemia nor hyperaminoacidemia alone were able to stimulate hepatic glucose production under these conditions. Our findings also agreed with a report by Yki-Jarvinen et al. (25) who found that infusion of acetate, a metabolite of many AAs, did not affect glucose disposal.

This study provided information only on total-body glucose disposal. Theoretically, it is conceivable that AAs inhibited glucose disposal in some insulin-sensitive tissues while increasing it to the same extent in others. However, we consider this highly unlikely. A more appealing reason regarding why there was no inhibition of total-body glucose disposal by AAs may have been the uptake of these two substrates by different organs. During euglycemic hyperinsulinemia, skeletal muscle has been shown to be responsible for ~85% of glucose disposal (26). In contrast, only ~27% of intravenously infused AAs (mostly branched-chain AAs) is taken up by muscle, with the remainder going to the liver (27). Therefore, infusion of a solution containing a large amount of branched-chain AAs would probably have produced more competition between AAs and glucose for transport into muscle than was observed in this study. In fact, Schwenk and Haymond (9) reported that infusion of leucine and isoleucine, two branched-chain AAs that are predominantly taken up by muscle, decreased forearm muscle glucose uptake, whereas Chang and Goldberg (7) showed that leucine inhibited glucose oxidation in rat skeletal muscle. The AA mixture used by Tessari et al. (11) contained ~40% more branched-chain AAs than our mixture and, because of this, may have produced some insulin resistance.

Furthermore, our results cannot rule out the possibility that AAs may have had inhibitory effects on intracellular carbohydrate utilization, i.e., on carbohydrate oxidation or storage. We were unable to use indirect calorimetry to determine carbohydrate oxidation and storage, because this technique cannot be used during AA infusions. Nevertheless, intracellular competition between glucose and AAs seems unlikely under conditions in which the bulk of these two fuels are taken up by different tissues, i.e., glucose by muscle and AAs by the liver. In conclusion, our data suggested that intravenously infused AAs, in contrast to FFAs, had no adverse effects on total-body glucose disposal and did not cause insulin resistance.

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