Effect of Arginine on Glucose Turnover and Plasma Free Fatty Acids in Normal Dogs

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SUMMARY

Arginine infusion is known to increase the plasma levels of both immunoreactive insulin and glucagon, while it changes the plasma glucose concentration only slightly. In order to clarify the metabolic consequences of glucagon and insulin mobilization induced by arginine, we investigated the effects of this amino acid on the turnover and plasma concentration of glucose, and on the plasma concentration of free fatty acids (FFA) in seven normal dogs. Infusion of arginine hydrochloride induced a biphasic release of insulin, which in turn increased the over-all rate of glucose utilization by 40 per cent. There was, however, only a slight increase in plasma glucose concentration (4 per cent) since the rate of glucose production increased to the same extent as the rate of glucose utilization. We have concluded that the biphasic pattern of insulin release not only reflects the secretory capacity of β cells, but also plays an essential metabolic role in maintaining a balance between the production and utilization of glucose during an arginine challenge. The observed increase in glucose production is attributed to the release of glucagon; this release is essential if normoglycemia is to be maintained. The concentration of FFA in plasma decreased by 50 per cent during infusion of the amino acid. In the dog, arginine infusion rapidly reverses the pattern of fuel utilization characteristic of fasting; the release of free fatty acids from fat depots is inhibited, while the turnover of glucose is enhanced even though normoglycemia is maintained. DIABETES *22*:537-43, July, 1973.

The finding that the amino acid leucine stimulated insulin release[1,2] prompted extensive investigation into the role of other amino acids in the regulation of hormone secretion. Arginine, one such widely studied amino acid, was found not only to release insulin,[2-7] but also to concurrently release glucagon.[4-6] Evidence for the stimulatory effect of arginine on glucagon and insulin secretion has now been obtained in man,[2-4] in dogs,[5] in the perfused dog pancreas,[6] in the perfused rat pancreas[7] and has been confirmed in isolated islets of Langerhans.[8,9] Arginine thus seems to have a role distinct from that of glucose in the regulation of endocrine pancreatic function, since the monosaccharide inhibits glucagon release while it stimulates insulin secretion.[6,8]

While the mechanism of action of arginine on α and β cells is now being probed,[10-12] and while the patterns of glucagon and insulin release in response to arginine have been extensively studied, the metabolic consequences of the simultaneous hormone mobilization have not yet been satisfactorily explored. It is known that arginine infusion is associated with the maintenance of normoglycemia in dogs,[5] and that it induces only a minor hyperglycemia in man,[2,3,13] but measurements of glucose concentration do not necessarily yield information concerning glucose dynamics. The fact that in dogs normoglycemia is maintained during arginine infusion, in spite of elevated insulin and glucagon levels, can be explained in either of two ways. Either the effects of the two hormones neutralize each other, and no increases in the rates of glucose production and utilization occur, or production and utilization of glucose increase synchronously thereby maintaining normoglycemia. Support for the second alternative was obtained from experiments using depancreatized dogs in which concurrent infusions of insulin and glucagon at rates of 2,880 μU./kg.-min. and 29 mμg./kg.-min., respectively, were found to be capable of maintaining normoglycemia even while they were inducing a 100 per cent increase in the turnover of glucose.[14]

The aim of these experiments, therefore, was to clarify the metabolic situation occurring during arginine infusion. This was done by measuring the turnover of glucose, using the method of primed tracer infusion, and by monitoring the plasma FFA concentrations during infusion of the amino acid into seven normal dogs.

MATERIALS AND METHODS

Surgical procedures and animals

Experiments were carried out on seven nonanesthetized female mongrel dogs which had been without food for eighteen hours. The animals were fed a high protein diet consisting of 200 gm. beef chunks (Dr. Bal-

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lard's Beef Chunks, Standard Brands Ltd.) and 200 gm. dog chow (Ralston Purina Ltd.) daily during the week prior to each experiment. During the same period of time, the dogs were trained to stand or lie quietly in a Pavlov harness.

On the day of the experiment, three vinyl catheters (Becton-Dickinson) were inserted into each dog which was under local anesthesia (1 per cent Xylocaine). Two catheters were pushed a short distance into each of the cephalic veins, and the third was introduced (after entry via the saphenous vein) into the inferior vena cava so that its tip was located below the point of entry of the hepatic veins. The cephalic cannulas were used for infusion of tracer glucose and arginine respectively; the saphenous cannula was used for blood withdrawal. Following the cannulations the animals were transferred to the Pavlov harness and left for sixty minutes prior to the start of the tracer infusion. At the end of each experiment the patency of all cannulas was verified and the final hematocrits (never below 0.35) were recorded.

Experimental design

All experiments consisted of two sequential ninety minute periods and a third seventy-five minute period. The primed tracer infusion was started at the beginning of the first period and continued throughout the experiment. Initial control values for all measured parameters were calculated from data obtained during the last forty minutes of the first period. Arginine hydrochloride (Matheson, Coileman and Bell; 290 mg. per milliliter water; pH 7.4) was infused at a rate of 12.5 mg./kg.-min. during the second period. The third period provided information about the re-establishment of the initial basal conditions. The tracer and arginine hydrochloride solutions were infused using Harvard and Sage infusion pumps respectively.

Tracer methods and calculations

The rates of endogenous (hepatic) glucose production ("rate of appearance," R_a) and utilization ("rate of disappearance," R_d) were determined using the method of primed tracer infusion, the calculations being carried out according to the method of Wall et al.[15] as simplified by De Bodo et al.[16] This tracer method is based on a single compartment analysis of glucose kinetics and assumes that rapid changes in the specific activity and concentration of glucose do not occur uniformly within the entire glucose pool. It was originally suggested[15,16] that the non-steady state term of the equation for R_a be multiplied by a correction factor (pool fraction) of 0.5 in order to compensate for non-uniform mixing. Subsequently however, Cowan and Hetenyi[17] estimated experimentally that the correct pool fraction for normal dogs was 0.65. This experimentally verified pool fraction (0.65) was therefore used in all of our calculations.

Furthermore, instead of using glucose concentrations and specific activities (SA) from two consecutive samples to generate the data necessary for the calculation of R_a and R_d, as was done previously,[15,16] values from three consecutive samples were used. Straight lines were fitted, using the method of least squares, to triplicates of both SA and glucose concentration. Once a fit was accomplished with one set of triplicates the first of the three values was dropped, the next sequential value was added and the subsequent fit was carried out (sliding fit). The equations of the lines thus generated were used to provide the information for the rate equations. The time boundaries of a particular R_a and R_d were defined to be the midpoints of the two time intervals in the triplicate. Use of this sliding fit technic minimized the fluctuations caused by random errors in the determination of the concentrations and specific activities of glucose.

The time of the priming tracer injection was taken as $t = 0$; R_a and R_d were not calculated prior to $t = 50$ minutes. The amount of tracer injected (1-C-14-glucose; New England Nuclear) equaled the amount infused in 110 minutes. All values of R_a and R_d were corrected for inaccuracies arising as a result of recirculation of the 14-C-label into newly formed glucose.[18] For our calculations we used a Poly-Com time-sharing computer system.

Processing of blood samples

The collection and immediate processing of blood samples have been described previously.[19] Glucose was isolated with the aid of an ion exchange resin (Bio-Rad Ag-2-X8) and its plasma concentration was subsequently determined using the method of Huggett and Nixon.[20] Plasma concentrations of FFA were measured according to the procedure of Dole and Meinertz,[21] the concentration of α-amino-nitrogen by the technic of Frame and Russell,[22] and the plasma immunoreactive insulin level by the method of Hales and Randle,[23] using the Amersham Searle Kit. All radioactivities were determined using liquid scintillation counting procedures and the specific activities of glucose were corrected for recirculation of the label according to the method of Reichard et al.[18]

RESULTS

The results depicted in the first two figures are from a representative experiment in which arginine was in-

fused into a normal dog. As shown in figure 1, the α-amino-nitrogen level rose throughout the period of arginine infusion and reached a level 220 per cent above the mean of the control period. The release of insulin in response to the arginine was biphasic. The plasma IRI level reached peaks 155 per cent and 54 per cent above the mean control values at five and seventy-five minutes respectively. The plasma FFA concentration fell throughout the period of arginine infusion, and was reduced by 53 per cent at its cessation.

Figure 2 shows the glucose data from the same experiment. The specific activity and concentration of glucose were stable during the control period, indicating the existence of a dynamic steady state; R_a and R_d were equivalent and normal, as was the plasma glucose concentration. During infusion of arginine the SA fell, suggesting an increase in glucose production by the liver. The calculated average value of R_a increased by 70 per cent during arginine infusion, and that of R_d by 56 per cent, but as a result of the continued balance between these rates, the concentration of plasma glucose rose by only 9 per cent.

The mean values of α-amino-nitrogen, plasma IRI, and FFA from the seven experiments are shown in figure 3, while the glucose data from each experiment are listed in table 1. As the figure and table show, the values of all parameters were normal during the initial control period and the mean changes induced by arginine infusion resemble very closely those seen in the previously described experiment. The average total increases in R_a and R_d during the entire period of arginine infusion in the seven dogs were 38 and 43 per

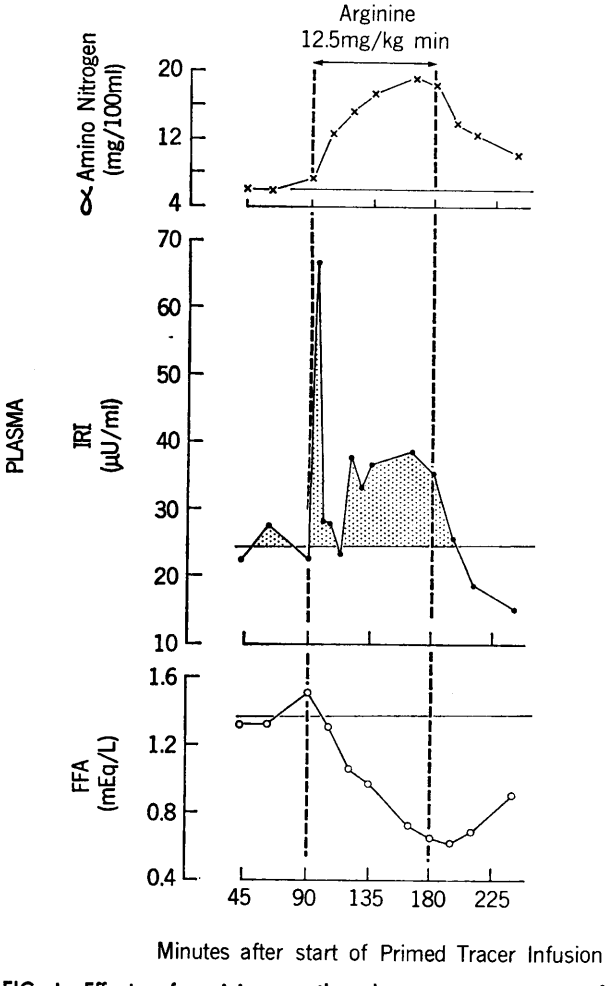

FIG. 1. Effects of arginine on the plasma concentrations of α-amino-nitrogen, immunoreactive insulin (IRI), and free fatty acids (FFA) in a normal dog.

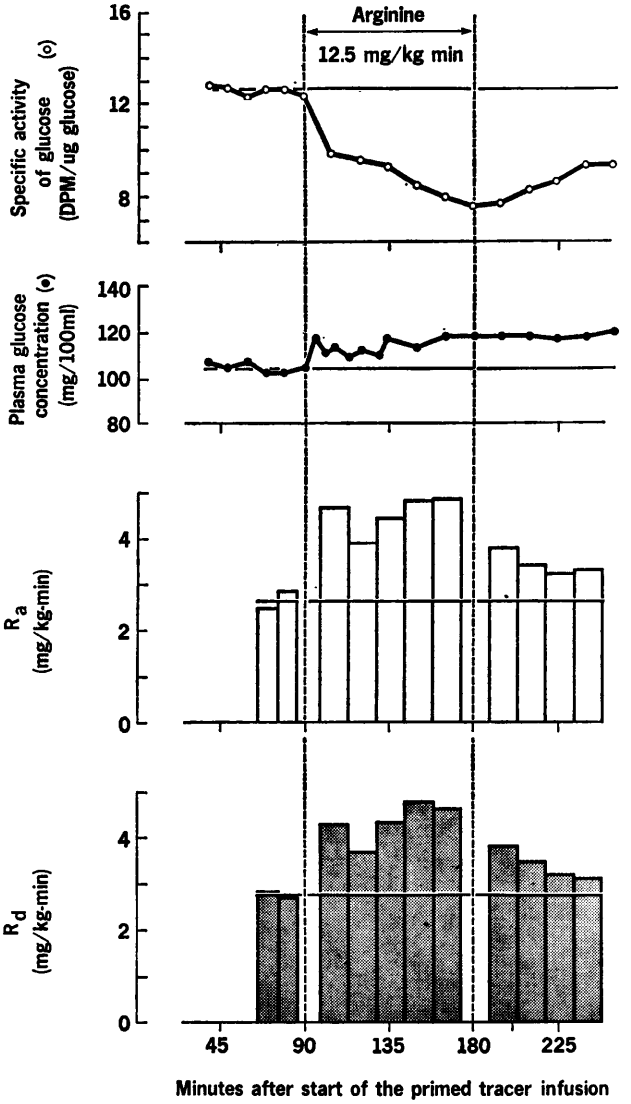

FIG. 2. Effects of arginine on the specific activity, concentration, and rates of appearance (production) and disappearance (utilization) of glucose in a normal dog.

cent respectively. Despite the significant increase in glucose turnover, the mean average glucose concentration rose by only 4 per cent (4 ± 2 mg./100 ml.). Unlike the relatively stable glucose level, the mean plasma FFA concentration fell significantly during arginine infusion.

During the recovery period the mean IRI concentration quickly returned to normal, the mean R_a and R_d values were slower in their decline, and the mean FFA concentration remained at its low level. The relatively slow re-establishment of the initial glucose homeostasis, and the reduced FFA level that persisted in spite of the rapidly normalized concentration of plasma insulin, might be explained by the experiments of Rasio et al.,[24] which indicated that insulin levels in the interstitial fluid compartment may remain elevated even when the concentration of insulin in plasma has become normal.

DISCUSSION

The main observation of these experiments was that the turnover of glucose increased significantly during arginine infusion, even though normoglycemia was maintained. This finding was particularly interesting because the rate of arginine infusion which we used was comparable to the rates used clinically in the arginine infusion test, and to those previously shown by others to stimulate insulin and glucagon release. The observed maintenance of normoglycemia can readily be explained by the synchrony of the increases in R_a and R_d and by the continued equivalence of the two rates. In view of this observation it does not appear that the effects of the concurrently released pancreatic hor-

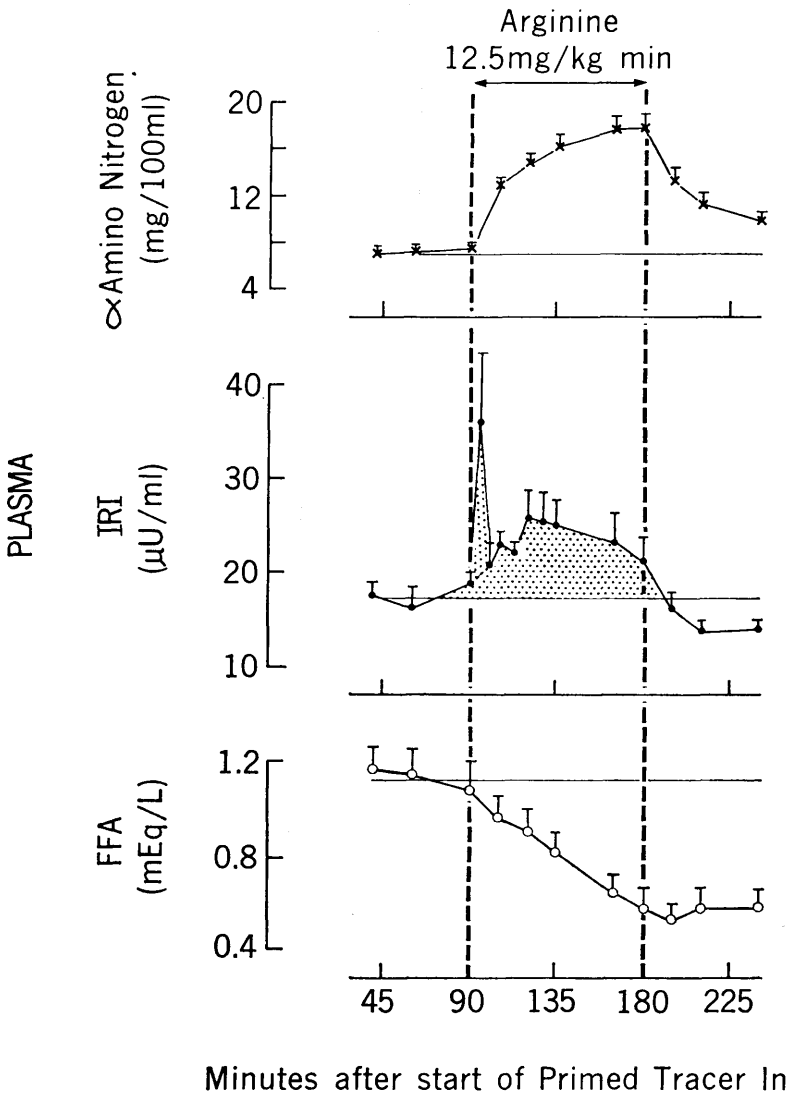

FIGURE 3

Effects of arginine on the mean plasma concentration of α-amino-nitrogen, immunoreactive insulin (IRI), and free fatty acids (FFA) in seven normal dogs. Paired tests were carried out between the initial control period means (each an average of three values), and the values at each subsequent time. A $p < .05$ was considered to be significant. All α-amino-nitrogen values obtained during the infusion of arginine and during the recovery period were significantly elevated. All mean IRI values obtained during arginine infusion, except the 100 and 113 minute values, were significantly increased, whereas all FFA values, except the one at 105 minutes, were significantly decreased. The FFA values remained significantly reduced during the recovery period, while the IRI values returned to normal within fifteen minutes.

TABLE 1

The concentrations and rates of production (R_a) and utilization (R_d) of glucose prior to, during and following infusion of arginine (12.5 mg./kg.-min.) into seven normal dogs

Dog #	Control Period Mean	Arginine Infusion Period											Postarginine Period				
Time:*		90	95	100	105	113	120	128	135	150	165	180	115	210	225	240	255
		Plasma Glucose (mg./100 ml.)															
1	102	101	—	—	104	—	105	—	102	96	104	96	105	107	107	110	106
2	103	102	—	—	102	—	102	—	110	101	97	98	102	102	93	102	96
3	105	108	109	108	110	108	110	113	109	101	103	108	108	100	99	95	93
4	102	104	110	109	110	98	106	106	111	99	103	106	105	105	108	101	100
5	104	104	117	111	113	109	112	110	117	113	118	118	118	118	117	118	120
6	103	102	108	97	104	96	102	97	106	103	112	109	111	107	106	109	104
7	95	102	94	94	111	109	107	—	111	105	110	107	108	112	111	110	111
Mean	102	103	108	104	108	104	106	106	109	103	107	106	108	107	106	106	104
S.E.M.	1	1	4	4	2	3	1	3	2	3	3	3	2	2	3	3	4
Time:*				97.5-112.5		112.5-127.5		127.5-142.5		142.5-157.5		157.5-172.5	187.5-202.5		202.5-217.5		217.5-232.5	232.5-247.5
		R_a (mg./kg.-min.)															
1	3.15			5.33		4.24		4.97		4.40		3.90	4.20		3.32		2.84	2.97
2	3.66			4.70		4.93		5.31		5.00		5.20	5.66		4.60		5.33	6.18
3	2.72			2.61		2.91		2.99		3.46		3.93	3.27		3.79		3.93	3.39
4	2.45			3.01		3.28		3.73		3.86		4.25	3.69		3.61		3.07	3.00
5	2.68			4.68		3.92		4.45		4.83		4.87	3.81		3.46		3.22	3.32
6	3.32			3.60		3.56		3.62		3.69		3.44	2.72		2.99		2.99	2.77
7	2.01			3.43		2.89		2.54		2.57		2.73	2.96		2.55		2.60	2.35
Mean	2.86			3.91		3.68		3.94		3.97		4.05	3.76		3.47		3.43	3.43
S.E.M.	0.21			0.38		0.28		0.38		0.32		0.32	0.37		0.24		0.35	0.48
		R_d (mg./kg.-min.)															
1	3.10			5.12		4.35		5.42		4.31		3.93	3.61		3.21		2.70	3.05
2	3.73			4.65		4.52		5.40		5.75		5.37	5.40		5.10		5.38	6.04
3	2.46			2.54		2.96		3.40		3.70		3.57	3.67		4.21		4.13	3.67
4	2.29			2.91		3.24		4.05		4.23		3.93	3.71		3.49		3.24	3.37
5	2.79			4.30		3.71		4.36		4.79		4.64	3.84		3.50		3.22	3.17
6	3.20			3.59		3.49		3.58		3.49		3.26	2.78		3.16		3.94	2.82
7	1.80			3.24		2.87		2.60		2.61		2.66	2.81		2.43		2.66	2.38
Mean	2.77			3.76		3.59		4.12		4.13		3.91	3.69		3.59		3.47	3.50
S.E.M.	0.24			0.36		0.24		0.39		0.38		0.34	0.33		0.32		0.37	0.45

R_a and R_d values obtained during the infusion of arginine and the first two values of the recovery period were significantly elevated, while only glucose values at 105, 120 and 135 minutes were significantly increased.

* Minutes of tracer infusion.

mones on R_a and R_d neutralize each other. Such a possibility was supported by the observation that insulin can inhibit the effect of glucagon on glucose production by the liver,[25,26] and by the suggestion of Rathgeb et al.[27] that glucagon can inhibit the stimulatory effect of mobilized insulin on peripheral glucose uptake. The latter suggestion is questioned, however, since in a recent study using depancreatized dogs maintained normoglycemic with a constant infusion of insulin we failed to detect any peripheral effect of glucagon on glucose utilization.[28] The ability of the normal dog to increase glucose turnover acutely while maintaining normoglycemia has been observed not only during arginine infusion, but also during exercise,[29] and during the infusion of glucagon.[14]

While the increase in glucose utilization observed during arginine infusion is clearly attributable to mobilized insulin, the cause of the synchronous increase in glucose production is less clear. It does not seem likely that arginine per se causes the increase in glucose production, since this amino acid is not a good gluconeogenic substrate,[30] and it has been shown that very little arginine is converted into glucose during an arginine infusion test.[13] Arginine is known to stimulate the release not only of insulin, but also of glucagon[4-6] and growth hormone.[31] While growth hormone does not affect glucose production acutely, glucagon has been shown to be a potent hormonal stimulus for both glycogenolysis and gluconeogenesis.[26] Previously we showed that glucagon could increase the rate of glucose production by the liver in the presence of either normal or elevated insulin concentrations.[14,28] It is likely, therefore, that glucagon is responsible for the increase in glucose production seen during arginine infusion.

The observation that arginine induces a biphasic pattern of insulin release has been well documented in man,[2,3] in dogs,[5] and in perfused rat[7] and dog pancreases.[6] Recent work in the dog,[5] and in the perfused dog pancreas,[6] suggests that arginine also induces a biphasic pattern of glucagon release. In our earlier experiments using depancreatized dogs[14] it was necessary to infuse insulin at a rate of 2,880 μU./kg.-min (twelve times the basal rate), in order to maintain normoglycemia in the face of an initial rate of glucagon infusion of 13 mμg./kg.-min. The high initial rate of insulin infusion was essential in order to increase R_d at a rate adequate to match the rapid increase in R_a brought about by glucagon. Continuation of the initial rate of insulin infusion, however, eventually resulted in hypoglycemia, since the rate of glucose utilization began to exceed the rate of glucose production. These experiments made use of a surgical procedure which allowed instantaneous replacement of a conscious dog's endogenous insulin secretion with an equivalent exogenous insulin infusion.[32,33] From our experiments we have therefore concluded that the effects of both the initially and the more slowly released insulin (biphasic response) are important in balancing the rapid and continued effects of glucagon on glucose production and are thereby important in maintaining normoglycemia during arginine infusion.

It seems probable, based on work recently reviewed by Unger,[4] that the small increase in the plasma α-amino-nitrogen level occurring as a result of protein ingestion induces a mobilization of insulin and glucagon similar to that resulting from our infusion of arginine. One would thus expect that during protein ingestion, as during arginine infusion, glucagon and insulin increase the turnover of glucose while maintaining normoglycemia, and thus transfer glucose from the liver to the periphery. Such a conclusion would fit well with the suggestion of Unger[4] that glucose represents an important source of energy for the protein synthetic processes occurring in peripheral tissues after protein ingestion.

The significant drop in FFA which we observed during arginine infusion is in agreement with earlier findings of others[2] and presumably reflects a decrease in the release of FFA from fat depots. Although we did not measure FFA turnover, this conclusion is possible since Armstrong et al.[34] demonstrated that the uptake of FFA by nonfat tissues is governed solely by the plasma FFA concentration. Thus a change in the FFA level reflects only a change in the rate of FFA release from fat stores. The most likely cause of the inhibition of FFA release is the elevated insulin concentration seen during arginine infusion. It is also possible, however, that the elevated growth hormone concentration[31] contributes to the decrease of FFA in plasma, since Sirek et al.[35] showed that growth hormone can decrease the FFA concentration acutely, independently of insulin.

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