Quantitative Study on the Potentiating Effect of Arginine on Glucose-induced Insulin Response in Healthy, Prediabetic, and Diabetic Subjects

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SUMMARY

Glucose dose-relations of the early and late insulin responses were established in control, prediabetic and mildly diabetic subjects by performing glucose infusions with varying doses in each subject. Our earlier demonstration that the dose-response curves for both the early and late insulin responses are shifted toward the high glucose region in prediabetes and diabetes was confirmed. In a second series of experiments, a thirty minute arginine infusion was given fifty minutes before the start of the glucose infusion. The insulin response to arginine was reduced in the prediabetics and diabetics. Plasma insulin returned to base line at the start of the glucose infusion in the controls and prediabetics, but not in the diabetics. The early insulin response to glucose infusion was markedly enhanced by pretreatment with arginine at all glucose doses used, both in the controls and the prediabetics, whereas the late response was not modified. The results in the patients with mild diabetes were not conclusive. In the controls and prediabetics, the glucose-insulin dose-response curves for the early response were steeper after pretreatment with arginine. The percentile increase in insulin response was fairly constant throughout the range of hyperglycemia induced. The insulinogenic index was increased by arginine with a factor of around 2 in controls as well as prediabetics, regardless of the dose of glucose administered.

These results, together with data reported previously by our group, indicate that arginine acts on insulin secretion by magnifying, at a constant rate, the insulinogenic signal of glucose. In prediabetics (and probably also in persons with slight diabetes), in whom the insulinogenic signal of glucose on the β-cell is diminished, arginine exerts quantitatively a similar degree of amplification. Therefore, it seems reasonable to assume that the mechanisms which mediate the amplifying effect of arginine in the β-cell are not deranged in prediabetes. DIABETES *23*:161-71, March, 1974.

The insulinogenic effect of amino acids has been amply demonstrated both in vivo[1] and in vitro,[2-5] but the mechanism by which amino acids stimulate pancreatic β-cells to release insulin still is not known. Hellman et al.[6] have shown that the rate of oxidation of alanine, arginine, and leucine in the islets of obese hyperglycemic mice does not correlate with the ability of these amino acids to stimulate insulin release. Furthermore, it has been reported that some artificial non-metabolizable amino acids stimulate insulin release,[7-10] whereas metabolites of, for example, leucine, are ineffective in this respect.[1] These experiments suggest that amino acids do not act as insulin releasers simply by serving as metabolic substrate for the pancreatic β-cells. We arrived at similar conclusions by studying the insulinogenic effect of arginine in man. Our data suggested that arginine may stimulate insulin release by modulating the insulinogenic signal evoked by glucose in the β-cell, rather than through primary actions on the insulin-releasing machinery of the cell.[11,12]

In the present study we have attempted to explore further the mode of action of arginine by investigating its effect on the dose kinetics of glucose-induced insulin release in groups of subjects demonstrating quantitative differences regarding the insulinogenic action of glucose, namely control subjects, healthy individuals with decreased insulin response to glucose (prediabetics), and patients with mild diabetes mellitus.

METHODS

A total of 120 experiments were performed on fourteen healthy volunteers with normal intravenous glucose tolerance and six patients with latent or manifest diabetes. Seven of the healthy subjects demonstrated decreased insulin response to intravenous glucose infusion, as judged by our criteria,[13] and were therefore considered to be prediabetics. The remaining seven had normal insulin response to glucose.

The group of diabetics consisted of three persons with latent disease (normal fasting blood glucose concentration and no glucosuria but decreased intraven-

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ous glucose tolerance), and three subjects with fasting hyperglycemia but without glucosuria. None of the subjects received diabetes treatment.

Data regarding the subjects are presented in table 1. None weighed more than 110 per cent of ideal.

All subjects were on a free diet containing about 300 gm. of carbohydrates. The experiments were performed early in the morning after an overnight fast. Each subject participated in six studies with at least a three day interval between the experiments, thus serving as his own control. Teflon catheters were placed in a brachial vein of each arm and kept patent with a slow drip of saline.

Glucose dose-response study. In three series of experiments the effect of different glucose loadings on insulin release was evaluated. Glucose was administered as a rapid intravenous injection followed by a constant infusion through a Bowman digital pump for sixty minutes. The following doses of glucose were used: (1) 100 mg./kg. body weight as rapid injection and 5 mg./kg. per minute as infusion; (2) 250 mg. and 10 mg., respectively; (3) 500 mg. and 20 mg., respectively; (4) 1,000 mg. and 40 mg., respectively. The glucose doses used in healthy subjects and prediabetics were (1), (3) and (4), and in diabetics (2), (3) and (4). A 25 per cent glucose solution was used for the rapid injection, while the glucose concentrations of the infusions were adjusted to fit the flow rates of the pump. Blood was drawn into heparinized tubes from the opposite brachial vein ten minutes and immediately before the beginning of the glucose load and 5, 10, 20, 30, 40, 50, 60, 80, 100 and 120 minutes after the start of the glucose injection.

Effect of arginine on glucose-induced insulin release. Fifty minutes before the glucose test, arginine was administered as a priming dose in the amount of 150 mg./kg. body weight followed by the constant infusion of 10 mg./kg. per minute over thirty minutes. Blood samples were drawn at −60, −50, −45, −40, −30, −20, −10, and 0 minutes. At 0 minute, glucose infusion tests were performed as described above.

The intravenous glucose tolerance test was performed and the k values calculated according to Ikkos and Luft.[14] In this laboratory, k values below 1.0 are considered indicative of disease.

Chemicals and analytical methods. L-arginine monochloride was obtained in a 10 per cent aqueous solution (Vitrum AB, Stockholm) and used at appropriate dilutions. Glucose was determined enzymatically in whole blood with a commercial glucose oxidase preparation (Kabi AB, Stockholm, Sweden). Insulin in plasma was measured radioimmunologically by the double antibody technic of Hales and Randle[15] using a commercial kit (Radiochemical Centre, Amersham, England). Conventional statistical methods were employed.[16] Results are presented as mean ± S.E.M.

TABLE 1

Data regarding subjects of the study

	Subjects	Sex	Age	Per cent of ideal body weight*	I.V. GTT k-value
Controls	1	F	34	95	2.94
	2	M	34	93	3.96
	3	M	28	92	2.72
	4	F	34	110	3.85
	5	M	25	90	2.35
	6	M	34	86	3.01
	7	F	25	98	1.80
Prediabetics	1	M	27	100	2.89
	2	M	33	88	1.07
	3	M	27	108	1.17
	4	M	26	100	1.73
	5	M	28	97	1.86
	6	M	31	87	1.82
	7	M	48	93	1.36
Mild diabetics	1	F	27	103	0.91
	2	M	22	84	0.66
	3	M	57	85	0.68
	4	M	42	103	0.51
	5	M	50	100	0.83
	6	M	21	100	0.84

*According to Documenta Geigy, 1960

RESULTS

Insulin response to arginine infusion. As shown in figure 1, administration of arginine increased the blood glucose concentration in control as well as in prediabetic and diabetic subjects, the highest blood glucose levels being reached twenty to thirty minutes after the beginning of the arginine infusion. In diabetic subjects blood glucose concentration was significantly higher than in controls during the entire experiment, whereas in the prediabetics significantly higher glucose levels were observed only thirty minutes and longer after the start of the infusion.

The basal insulin level was significantly higher in diabetics than in controls (25 ± 2.5 vs. 18 ± 1.1 μU./ml., $P < 0.025$). Arginine induced a biphasic insulin release in all three groups of subjects. Plasma insulin increased sharply, a peak value being reached at five minutes. Both in the prediabetic and diabetic groups a somewhat delayed insulin curve was observed. In prediabetics, arginine induced significantly

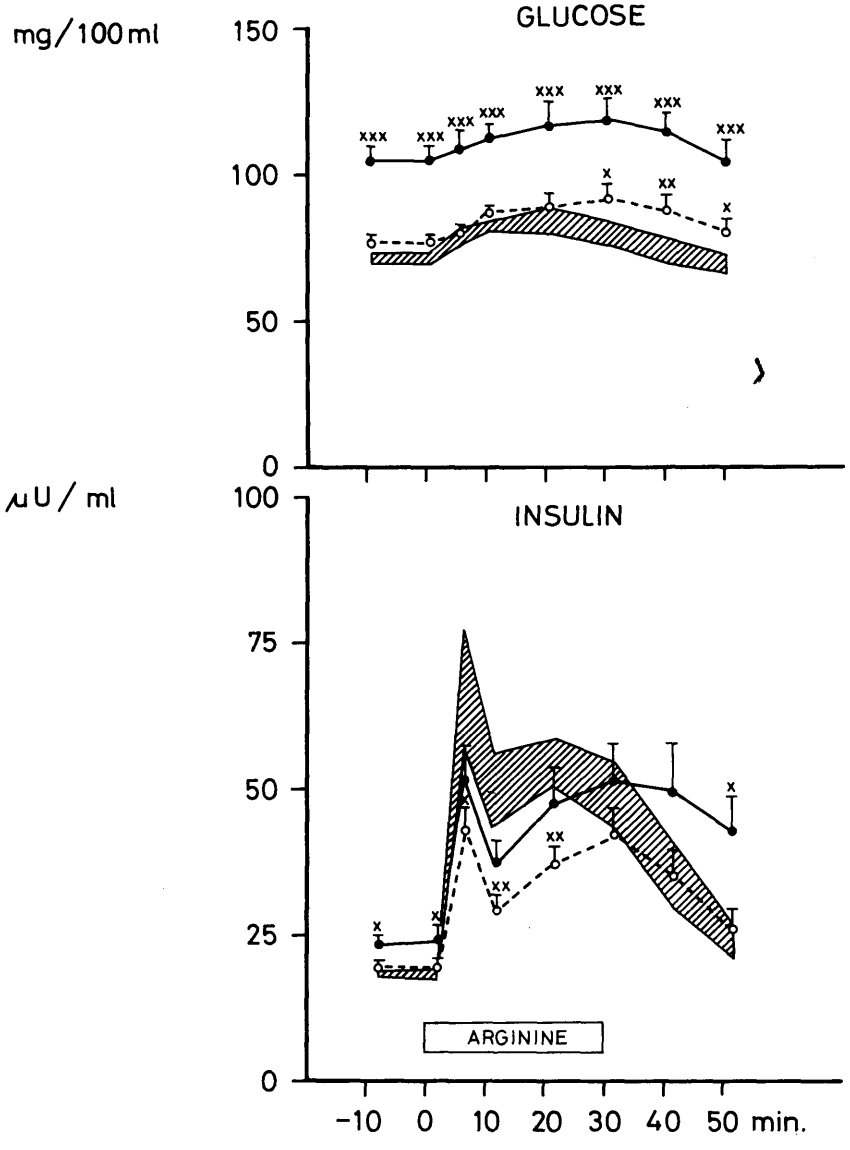

FIGURE 1

Effect of arginine on insulin release in control (n=7), prediabetic (n=7), and diabetic (n=6) subjects. Mean of three separate experiments on each subject. The shadowed area denotes Mean ± S.E.M. in the control group. •, curve of the diabetics; o, curve of prediabetic subjects. Vertical bars denote S.E.M. x $P < 0.05$, xx $P < 0.01$, xxx $P < 0.001$

lower insulin release during the first twenty minutes of the infusion ($P < 0.05$ at five minutes; $P < 0.01$ at ten and twenty minutes). In the diabetic group the initial insulin peak was only slightly decreased ($P > 0.05$). However, if the insulin response above the fasting levels is considered, the responses at five and ten minutes were significantly reduced in the diabetics as well ($P < 0.05$ and $P < 0.025$, respectively). When the arginine infusion was discontinued, plasma insulin decreased and returned to the basal level at fifty minutes in controls and prediabetics. On the other hand, the insulin levels were still elevated in diabetics at this time.

Effect of arginine preinfusion on glucose-induced insulin release in control subjects. As shown in figure 2, a thirty-minute arginine infusion, initiated fifty minutes before the glucose test, was followed by the usual insulin response. When glucose infusion was started at zero minutes, plasma insulin, which had returned to the fasting level, rose dramatically. Comparison with control experiments show that the initial insulin response was much smaller when glucose was not preceded by the arginine infusion, although the blood glucose levels were roughly of the same magnitude in both series of experiments (table 2). In contrast, the later insulin response was not stimulated by arginine preinfusion (table 2).

Effect of arginine preinfusion on glucose-induced insulin

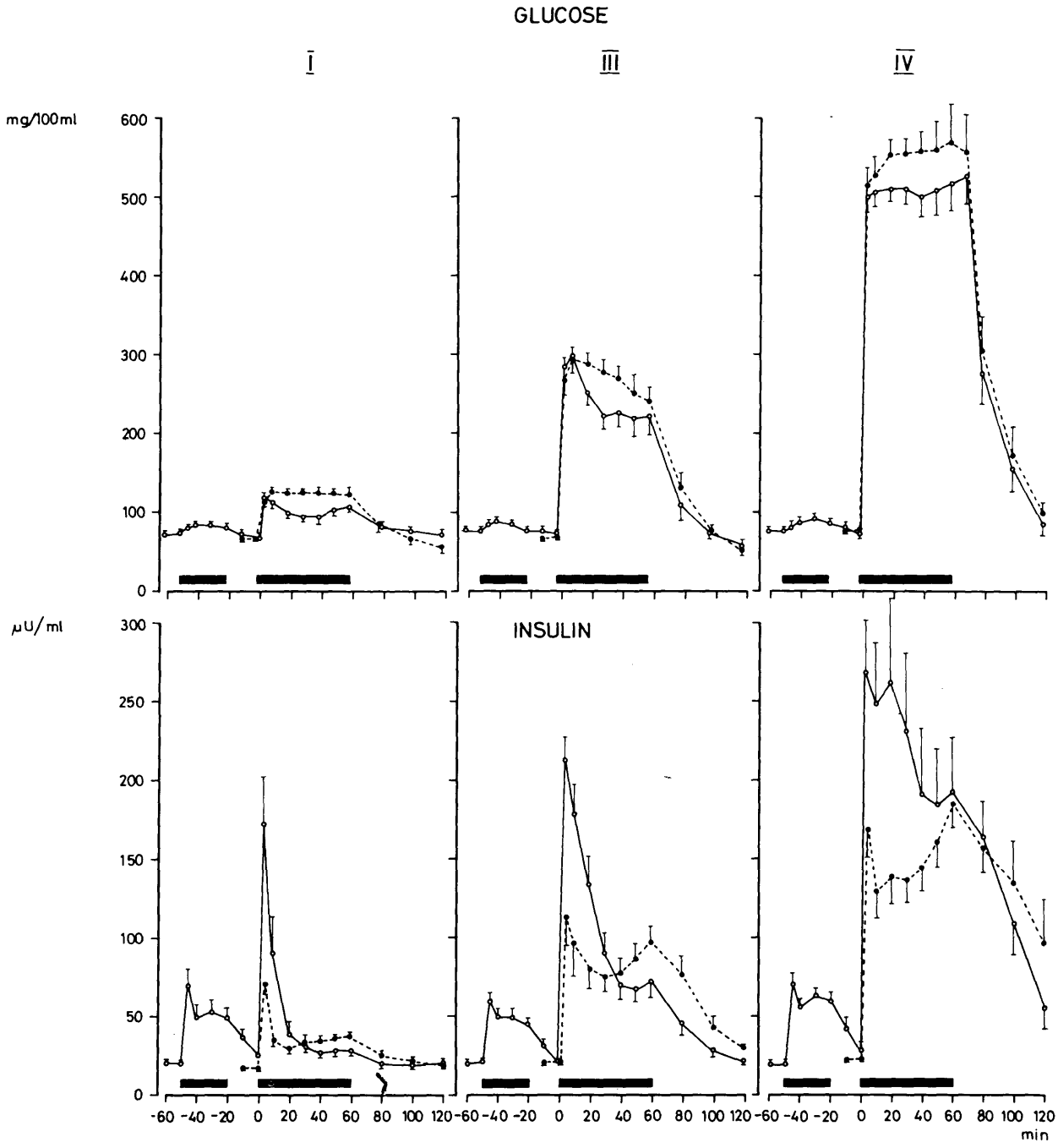

FIG. 2. Effect of arginine on the plasma insulin response to varying hyperglycemic stimuli in control subjects. Arginine was administered between -50 and -20 minutes and glucose between 0 and 60 minutes. The Roman numbers refer to the various glucose doses used (see Methods). •---•, effect of glucose infusion alone; o---o effect of combined arginine and glucose infusions.

release in prediabetics and diabetics. Glucose infusion, independently of the dose given, induced a lower insulin response in prediabetic and diabetic subjects than in controls (figures 3 and 4).

Arginine preinfusion enhanced markedly the defective insulin response to glucose in the prediabetic subjects, the response resembling that obtained in the controls by glucose alone (table 2). Also, in this

TABLE 2

Effect of varying hyperglycemic stimuli on early (ten minute) and late (sixty minute) insulin responses with and without arginine preinfusion

	Group	Glucose* loads	Blood glucose concentration (mg./100 ml.)			Insulin response above fasting level (μU./ml.)		
			Glucose infusion	Arginine and glucose infusion	p†	Glucose infusion	Arginine and glucose infusion	p†
EARLY RESPONSE	Controls	1	126.1 ± 5.2	116.5 ± 6.7	N.S.	18.0 ± 0.6	54.9 ± 3.2	<0.001
		3	291.2 ± 15.7	294.0 ± 9.8	N.S.	75.7 ± 3.5	142.1 ± 2.7	<0.001
		4	524.7 ± 21.8	503.7 ± 18.9	N.S.	107.6 ± 2.9	220.9 ± 6.2	<0.001
	Prediabetics	1	143.1 ± 7.6	133.6 ± 5.0	N.S.	1.8 ± 0.3	13.6 ± 0.3	<0.001
		3	330.7 ± 16.5	309.4 ± 7.5	N.S.	14.0 ± 0.2	50.6 ± 1.1	<0.001
		4	561.4 ± 27.8	533.7 ± 12.9	N.S.	33.6 ± 1.5	71.5 ± 2.0	<0.001
	Diabetics	2	230.3 ± 10.6	220.2 ± 7.7	N.S.	3.2 ± 0.3	11.3 ± 1.1	N.S.
		3	357.0 ± 27.2	371.2 ± 10.1	N.S.	5.5 ± 0.7	4.2 ± 1.1	N.S.
		4	581.8 ± 31.0	570.0 ± 17.7	N.S.	0.5 ± 1.7	12.2 ± 2.8	N.S.
LATE RESPONSE	Controls	1	121.7 ± 7.0	106.7 ± 5.3	N.S.	18.0 ± 3.0	7.9 ± 2.9	<0.05
		3	239.4 ± 13.7	220.4 ± 23.5	N.S.	68.3 ± 11.5	50.5 ± 11.2	N.S.
		4	554.0 ± 47.6	522.7 ± 35.0	N.S.	162.4 ± 16.2	176.4 ± 32.1	N.S.
	Prediabetics	1	165.0 ± 6.9	141.7 ± 9.2	<0.05	11.8 ± 2.7	7.7 ± 1.8	N.S.
		3	401.0 ± 24.9	312.6 ± 24.1	<0.05	63.0 ± 16.8	65.6 ± 11.3	N.S.
		4	653.7 ± 50.3	581.14 ± 37.5	N.S.	144.9 ± 23.4	105.6 ± 14.3	N.S.
	Diabetics	2	305.17 ± 4.4	276.17 ± 13.4	N.S.	12.7 ± 2.4	29.3 ± 7.8	<0.05
		3	546.0 ± 23.0	475.5 ± 23.0	<0.02	29.8 ± 4.1	39.3 ± 5.9	<0.05
		4	914.0 ± 4.3	808.7 ± 40.25	<0.05	54.8 ± 6.8	96.0 ± 30.1	N.S.

* See Methods
† Paired differences

group, no potentiation of the late response was observed (table 2). In the diabetics, the glucose infusion alone had only a slight initial effect on insulin release even when marked hyperglycemia was induced (table 2). Pretreatment with arginine had only a minor effect on the initial insulin response, while the late response—in the presence of lower blood glucose levels—seemed to be somewhat enhanced (table 2).

In figure 5, the results of the above glucose infusions with and without arginine pretreatment are presented in terms of the dose-response relation between the blood glucose concentration (logarithmic scale on the x axis) and the increase in plasma insulin (linear scale on the y axis). The ten minute values were chosen as representative of the early insulin response (figure 5A). It is apparent from the figure that arginine increased the slope of the dose-response curves in controls as well as in prediabetic subjects. It can be interpolated from the curves that, in controls, arginine potentiated the glucose effect by about 200 per cent in the blood glucose range of 200 to 500 mg. per 100 ml., whereas in prediabetics the effect was somewhat higher (270 to 300 per cent) in the same range of hyperglycemia. Arginine had no effect on initial insulin response in diabetics (figure 5A).

The glucose-insulin dose relationships for the late insulin response (sixty minutes) are presented in figure 5B. The figure illustrates clearly that the dose-response curves in prediabetics and diabetics are gradually shifted to the right of that of the control group. Pretreatment with arginine did not significantly influence the glucose-insulin relationship in the control and prediabetic subjects. In diabetics, arginine pretreatment seemed to have a slight potentiating action on the late insulin response, as apparent from the somewhat higher slope of the dose-response curve (figure 5B).

DISCUSSION

In spite of the considerable investigative effort of the last decade, the precise mode of action of different insulinogogues—physiologic or pharmacologic—is still not known. During recent years, work from our laboratory[17,18] and elsewhere,[19] has focused attention on the possibility that glucose may induce insulin release not primarily through its metabolism but by interacting with a specific β-cell membrane receptor which initiates an insulinogenic signal. The nature of this insulinogenic signal is obscure, but it has been

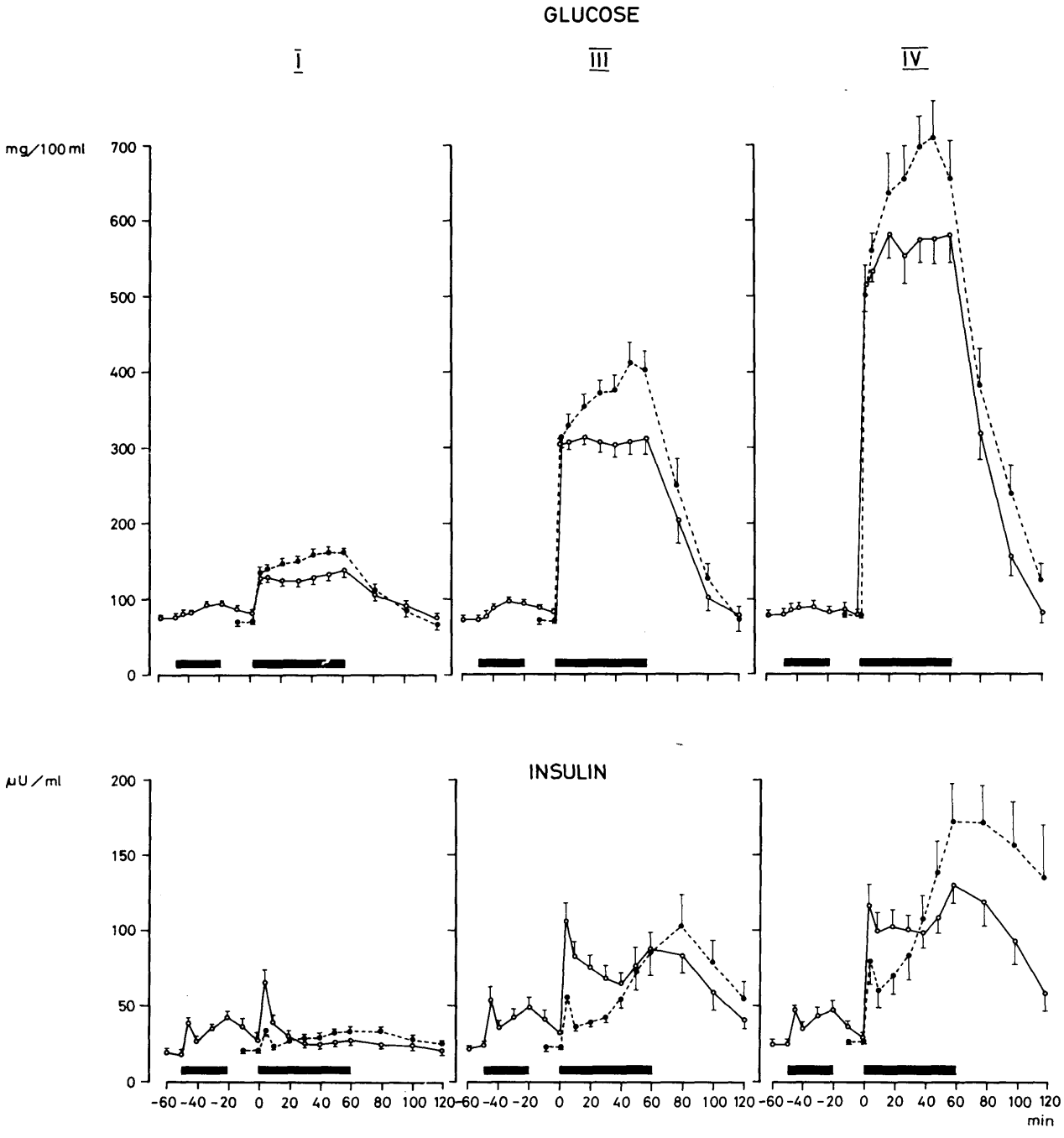

FIG. 3. Effect of arginine on the plasma insulin response to varying hyperglycemic stimuli in prediabetic subjects. Legend as in figure 2.

suggested that cyclic AMP[17] or calcium ions[20] or both may play a role in its transmission in the β-cell.

We have recently submitted data[11,12] indicating that another physiologic insulinogogue, arginine, may exert its action by modulating the signal elicited by glucose in the β-cell, rather than by a direct, independent effect of its own on the insulin-releasing machinery. We suggested that arginine probably interacted with the glucose signal early during the chain of events which transmits the stimulatory effect of

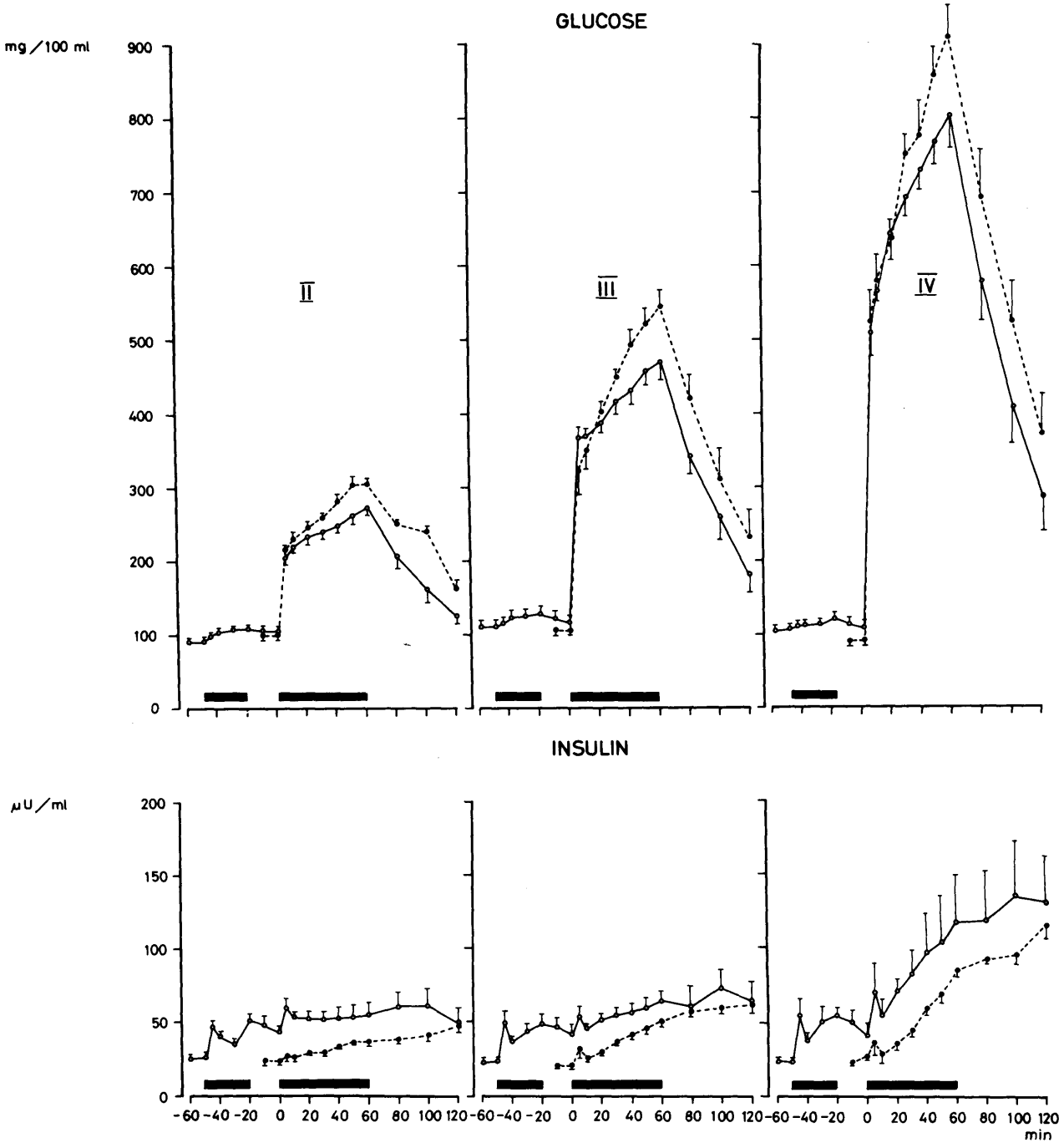

FIG. 4. Effect of arginine on the plasma insulin response to varying hyperglycemic stimuli in diabetic subjects. Legend as in figure 2.

glucose from the glucose receptor to the actual site in the β-cell where release of insulin occurs.[11,12] The hypothesis was based on the following findings: hypoglycemia inhibited, whereas hyperglycemia stimulated, arginine-induced insulin release;[11] under similar experimental conditions arginine had no effect on insulin release induced by glucagon;[12] in experiments where hyperglycemia was achieved through epinephrine infusion, no synergism could be observed between arginine and glucose on insulin secretion,[11]

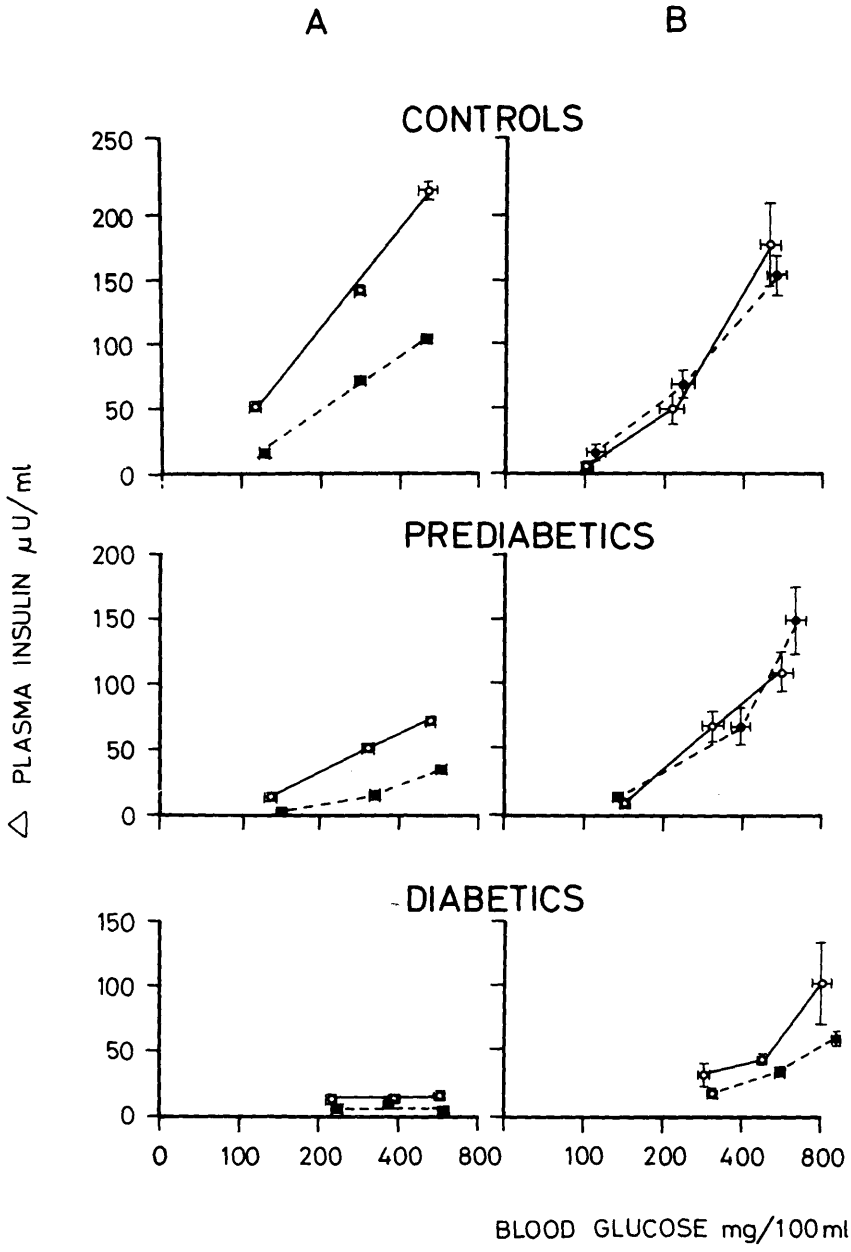

FIGURE 5

The glucose-insulin dose-response curves for the early (ten minute, "A") and late (sixty minute, "B") phases of insulin release during glucose infusion. ●---●, responses to glucose alone; o---o, responses to arginine + glucose infusion.

indicating that the synergism between arginine and glucose appears only when glucose itself is able to elicit insulin release; finally, pretreatment with aminophylline potentiated the insulinogenic effect of arginine.[12] In this connection it should be pointed out that Floyd et al.[21] demonstrated synergism between the insulinogenic effects of arginine and glucose.

The present experiments were undertaken with the objective of clarifying the quantitative aspects of the suggested interaction between arginine and glucose.

The experiments were designed so as to dissociate insulin release due to arginine and to glucose. Since, at the time of administration of glucose, plasma insulin had returned to the base line after having been stimulated by arginine (the group of diabetics excepted), the experimental design permitted demonstration of pure synergism between the two agents.

The blood levels of arginine were not measured in our experiments. However, it has been shown recently[22] that, when 0.5 gm./kg. of arginine is infused over thirty minutes (corresponding roughly to the dose used by us), plasma arginine increased from

the basal level of 0.07 to a peak value of 7.4 mM. at the end of the infusion, then returning slowly to the base line. It can be deduced from these data that, at the time of glucose administration in our experiments, plasma arginine concentration would be around 4 mM.

Potentiation by arginine of the glucose effect on initial insulin release was impressive in the control group. The quantitative evaluation of this potentiation demonstrates that arginine enhances the glucose effect percentilely at a constant rate, regardless of the magnitude of the glucose stimulus used, at least in the blood glucose range of 200 to 500 mg./100 ml. This constant effect of arginine is emphasized in table 3, where the ratios between the insulinogenic indexes in the presence and absence of the amino acid are presented. It is clearly seen from the table that arginine, at all glucose doses used, increased the insulinogenic index by a factor of around 2. The present findings, taken together with our earlier work,[11,12] indicate that arginine is a multiplicative potentiator of glucose-induced insulin release. It may be suggested that the amino acid acts by amplifying the signal induced by glucose in the β-cell, though the site of the interaction remains obscure. However, it cannot be excluded that arginine might stimulate insulin release by activating specific arginine-sensitive receptors.

Our data are not in accord with the report of Levin et al.[23] on isolated perfused rat pancreas, according to which arginine potentiated insulin release induced by lower glucose concentrations (70 to 150 mg./100 ml.), but had no effect on higher glucose loads (300 or 500 mg./100 ml.). It is possible that, in the perfused rat pancreas preparation used in those experiments, the secretory capacity of the pancreas is saturated when the glucose stimulus is as high as 300 to 500 mg./100 ml. Obviously, species differences also have to be borne in mind.

In our studies, the late insulin response to glucose was not modified in the controls (figure 5B), probably due to the further decrease in plasma arginine concentration.[22] Similarly, Levin et al.[24] did not observe potentiation of the insulin response to glucose in experiments in which the infusion of 20 gm. of arginine was followed by glucose administration thirty minutes later, when the plasma concentration of arginine had probably decreased below a critical level.

If one regards—as we do—arginine as an agent that modifies insulin release by amplification of the glucose signal in the β-cell, it becomes of considerable interest to evaluate its action in prediabetics and diabetics, since β-cells in these subjects do not respond adequately to glucose.[13,18] We have previously suggested that this inability to respond to glucose is probably caused by a defective glucose receptor in the β-cell, other cell functions being intact.[17,25] Our recent demonstration that the glucose-insulin dose-response curves in prediabetic and diabetic individuals are parallelly shifted to the right of the normal one,[18] provides experimental support for this idea. The effect of arginine on glucose-induced insulin release in prediabetic subjects, as observed in the present investigation, could be predicted from the above hypotheses. Arginine preinfusion potentiated the initial insulin response to glucose infusion at all glucose doses used, the degree of potentiation being fairly constant (figure 5A and table 3). As seen in table 3, the insulinogenic index was enhanced by arginine to the same extent in the prediabetics as in the controls. It can be concluded, therefore, that the mechanisms in the β-cell which mediate the potentiatory action of arginine on to the glucose signal are not deranged in prediabetic subjects. However, the total amount of insulin secreted by these subjects after stimulation with arginine plus glucose is less than normal, since the multiplica-

TABLE 3

Effect of arginine preinfusion on insulinogenic indexes* obtained ten minutes after glucose administration

Glucose dose†	Controls			Prediabetics		
	No arginine	Arginine	Ratio: $\frac{\text{arg}}{\text{no arg}}$	No arginine	Arginine	Ratio: $\frac{\text{arg}}{\text{no arg}}$
1	0.27 ± 0.03	0.46 ± 0.8	1.8 ± 0.3	0.16 ± 0.02	0.29 ± 0.03	1.9 ± 0.2
3	0.34 ± 0.07	0.67 ± 0.07	2.0 ± 0.2	0.10 ± 0.01	0.27 ± 0.04	2.8 ± 0.5
4	0.24 ± 0.04	0.49 ± 0.07	2.1 ± 0.3	0.09 ± 0.02	0.18 ± 0.03	2.0 ± 0.4

* Insulinogenic index = $\frac{\text{plasma insulin } (\mu\text{U. per ml.})}{\text{blood glucose (mg. per 100 ml.)}}$

† See Methods

tive action of arginine will be exerted on a reduced glucose signal. Recently, we studied the effect of oral glucose loads on insulin release in control, prediabetic and diabetic subjects and arrived at similar conclusions, namely that the potentiatory effect of gut factors on glucose-induced insulin release was not deranged in subjects with the diabetes syndrome.[26]

Also, the insulin response to arginine infusion itself was reduced in the prediabetics (figure 1). It can be assumed that the very high plasma arginine levels obtained by injection and perfusion of the amino acid strongly potentiate the effect of the basal blood glucose concentration on the β-cell. Therefore, the reasoning outlined above may be applied to explain the reduced response to arginine infusion in prediabetes.

Insulin response to glucose is further decreased in diabetic subjects,[13] and in the present series no early insulin response could be obtained. In other words, the insulinogenic signal of glucose was minimal in our patients. It is not surprising, therefore, that the arginine levels prevailing at the time of glucose administration were not sufficient to potentiate this signal to the extent of causing significant insulin release. However, higher concentrations of arginine—as achieved during infusion of the amino acid—could elicit insulin secretion, albeit less than normal; the observation is in agreement with the data of Floyd et al.[27] and Kipnis.[28] When comparing the response of the diabetic with the prediabetic patients (figure 1) it has to be taken into consideration that the blood glucose levels were higher in the former subjects. The response in the diabetic group thus probably corresponds to a combination of the basal state plus some degree of hyperglycemic stimulation.

In diabetics the late insulin response to glucose is usually less impaired than the early one.[13,18] That observation was made in the present studies also. Our finding that the late response was somewhat potentiated by arginine in the diabetics, but not in the controls and prediabetics, is difficult to explain. Since plasma arginine was not measured, we cannot exclude the possibility of a prolonged half-life of the amino acid in our diabetic subjects.

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