Hyperinsulinemia Decreases Second-Phase But Not First-Phase Arginine-Induced Insulin Release in Humans

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The aim of this study was to investigate the effect of hyperinsulinemia on the first and second phase of arginine-induced insulin release in humans. Seven healthy subjects underwent three studies (lasting 360 min): a control study using saline infusion and two euglycemic clamps using a low-dose (0.33 mU · kg^{-1} · min^{-1}) and a high-dose (1.20 mU · kg^{-1} · min^{-1}) insulin infusion. After a 3-h equilibration period, arginine (25 g) was infused for 30 min, and insulin and C-peptide responses to arginine were followed for 180 min. At the end of the equilibration period, before arginine administration, steady-state insulin levels were (means ± SE) 60.0 ± 2.4, 165.6 ± 1.8, and 455.4 ± 7.8 pmol/l during saline, low-dose, and high-dose insulin infusions, respectively. The time course of insulin release during the arginine test was calculated from C-peptide concentrations by using C-peptide kinetic modeling and deconvolution. In particular, first-phase and second-phase insulin response was obtained by integrating the time course of the insulin release during either the first 5 min or the following 40 min of the arginine test, respectively. Whereas first-phase insulin release was independent of any effect induced by either insulin infusion, second-phase insulin release was reduced in a similar degree by both insulin infusion doses. First phase was 75.5 ± 10.1, 73.7 ± 12.8, and 73.4 ± 10.3 pmol/kg, whereas second phase was 266.1 ± 46.0, 143.1 ± 33.5, and 133.0 ± 30.2 pmol/kg for saline, low-dose, and high-dose insulin infusions, respectively. We conclude that second-phase, but not first-phase, arginine-induced insulin release is modulated by the pre-stimulus insulin levels. In addition, the inhibitory effect exerted by insulin on second-phase insulin response to arginine appears to be maximized at insulin levels only four times basal. ***Diabetes*** **1157–1163, 1994**

Several studies have documented that exogenously induced hyperinsulinemia is able to inhibit endogenous insulin release at normoglycemia (1–6). On the other hand, little is known about the feedback effect of insulin on the first and second phase of insulin response to a stimulus. Liljenquist et al. (7) and Asplin et al. (8) have shown that the presence of exogenously induced hyperinsulinemia reduces the magnitude of insulin response to either glucose (7) or arginine (8) in humans. In such studies, insulin release was only assessed by measurements of either insulin (8) or C-peptide in peripheral blood (7). However, insulin release cannot be accurately evaluated from peripheral concentrations of insulin because of the large and variable hepatic extraction of insulin (9–11). Furthermore, although C-peptide is cosecreted with insulin on an equimolar basis and its extraction by the liver is negligible, C-peptide peripheral levels do not reflect the actual time course of insulin release because of the long half-life of the peptide (12). For these reasons, the modalities of insulin feedback effect on the two phases of stimulated insulin release remain to be clarified.

The aim of this study was to quantify the acute effects of prolonged, physiological hyperinsulinemia on arginine-stimulated first- and second-phase insulin response to arginine in normal subjects. Arginine was chosen to stimulate not only insulin but also glucagon and somatostatin release (13–16). Our results indicate that second-phase, but not first-phase, insulin release during arginine infusion is modulated by the pre-stimulus insulin levels. In addition, suppression of the second phase by insulin is maximized at insulin levels only four times basal.

RESEARCH DESIGN AND METHODS

Experimental protocol. The protocol of the study was approved by the local ethic committee, and informed consent was obtained from each participant. Seven healthy men were studied (20–26 years of age, body mass index [BMI] 22.5 ± 0.5 kg/m^2). Each subject underwent three studies (lasting 360 min) in random order: a control study using saline infusion and two euglycemic clamps using low-dose (0.33 mU · kg^{-1} · min^{-1}) and high-dose (1.20 mU · kg^{-1} · min^{-1}) insulin infusion (Actrapid MC, Novo Nordisk, Bagsværd, Denmark). Specifically, the procedure was as follows: at 0800, a 20-gauge plastic cannula (Abbocath T, Abbocat, Ireland, SLIG, Ireland) was inserted in a dorsal vein of one hand in a retrograde position, and the hand was placed in a thermoregulated plexiglass box maintained at 55°C to permit sampling of arterialized blood. The antecubital vein was also cannulated and used for pump-assisted infusions (saline or insulin). During the insulin infusions, blood glucose was maintained at the basal level by a variable infusion of 33% glucose. The infusion was adjusted according to glucose levels determined every 5 min using a glucose analyzer (YSI, Yellow Springs, OH). Each 33% glucose bag contained potassium (6 mEq/l for low-insulin infusion; 20 mEq/l for high-insulin infusion) to prevent hypokalemia. In addition, heparin (100 U every 60 or 30 min, respectively) was injected intravenously to prevent insulin-induced drops in free fatty acids (FFAs) during both insulin infusions. After a 3-h equilibration period, at time 0, arginine (25 g) was infused for 30 min. Plasma C-peptide, insulin, glucagon, and somatostatin were measured at the following time points

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BMI, body mass index; FFA, free fatty acid; CPR(t), C-peptide release; CV, coefficient of variation; AUC, area under the curve.

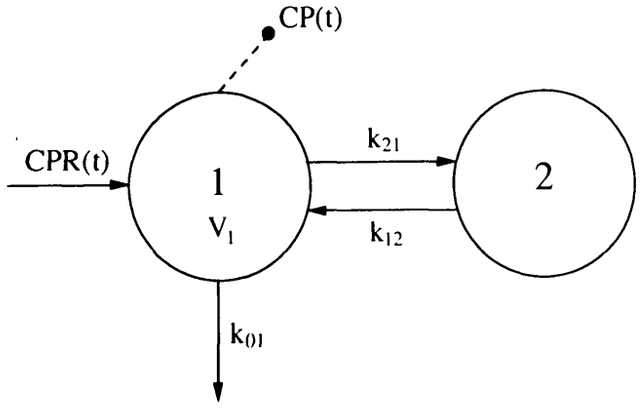

FIG. 1. The two-compartment model of C-peptide kinetics (12,17,18). The model assumes that CPR(t) occurs in the accessible pool (compartment 1), and then C-peptide distributes in a peripheral compartment (compartment 2). Sampling of C-peptide concentration, CP(t), occurs in the accessible pool of volume V_1. Parameters k_{01}, k_{21}, and k_{12} are rate constants (min^{-1}).

during the arginine test: 1, 3, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 min. During the first part of the arginine test (~50–60 min), the glucose infusion used during the two insulin studies was not varied. It was maintained at a constant rate achieved at the end of the 3-h equilibration period so that the blood glucose was free to increase in a similar way in each study in response to increased glucagon levels. Blood glucose returned to the pretest level ~20–30 min after the end of the arginine infusion and was thereafter clamped until the end of the study.

Kinetic analysis. Eaton et al. (17) developed a deconvolution method for estimating insulin release from peripheral C-peptide concentrations. This method uses a mean two-compartment model of C-peptide kinetics identified from data obtained in diabetic subjects (18) and reconstructs insulin release in each individual from a spline fit of the subject's peripheral C-peptide concentrations. The model (Fig. 1) assumes that C-peptide release, CPR(t), occurs in the accessible pool (compartment 1) and then C-peptide distributes in a peripheral compartment (compartment 2). Sampling of C-peptide concentration, CP(t) occurs in the accessible pool of volume V_1. Parameters k_{01},k_{21},k_{12} are rate constants (min^{-1}). This deconvolution approach has been subsequently refined by Polonsky et al. (19), who proposed a two-step strategy for the estimation of insulin release. First, the C-peptide model (and thus the set of model parameters V_1,k_{01},k_{21},k_{12}) is identified in each individual from the C-peptide decay curve following an intravenous bolus of the peptide. Second, the C-peptide model is used to reconstruct the time course of insulin release from a spline fit of C-peptide concentrations measured during the study. Recently, Van Cauter et al. (20) have shown that the parameters of the C-peptide model can be individualized on the basis of the clinical characteristics of the subjects without a significant loss of accuracy. We adopted this method to determine the model parameters in each subject. Table 1 shows the clinical characteristics of the subjects required for parameter individualization and the model parameters obtained using the procedure described previously (20).

Assays. All samples were assayed in one assay for insulin, C-peptide, pancreatic glucagon, and somatostatin content by radioimmunoassay. The intra-assay coefficients of variation (CVs) for insulin, C-peptide, glucagon, and somatostatin were 3.0, 3.0, 4.5, and 10%, respectively. Plasma FFAs (intra-assay CV 3.0%) were assayed using an automated fluorimetric technique adapted to Cobas Fara (Roche, Basel, Switzerland). Plasma calcium levels were determined by titration against methylthymol blue, and plasma potassium levels were determined by flame photometry.

Statistical analysis. Each variable was expressed as mean ± SE at each time interval. During the arginine test, the incremental area under the curve (ΔAUC) for insulin (first phase: 0–10 min; second phase: 10–75 min), C-peptide (first phase: 0–10 min; second phase: 10–75 min), glucagon (0–90 min), and somatostatin (first phase: 0–20 min; second phase: 20–120 min) was calculated by the trapezoidal rule. Comparisons between experiments were made on ΔAUCs by means of two-way analysis of variance followed by the paired Student's t test.

RESULTS

Pre-arginine period. All the hormonal and metabolic parameters studied were not different in the 30-min basal period before the initiation of the three studies. During the 3-h equilibration period, blood glucose levels were successfully clamped, with CVs <5.0%. Insulin concentration increased by two- to threefold and by six- to sevenfold during low- and high-insulin infusions when compared with saline infusion (Fig. 2*B*). During this period, C-peptide concentrations of both insulin-infusion doses remained significantly lower than the C-peptide concentration of the control study in the last 30 min of the equilibration period ($P < 0.05$), but there was no significant difference between them. Saline and both insulin infusions did not significantly change plasma levels of glucagon, somatostatin, FFA, potassium, and calcium (Fig. 3 and Table 2).

The time courses of insulin release (in staircase fashion) during the 3-h equilibration period for saline, low-insulin, and high-insulin infusion studies are shown in Fig. 4. During the two insulin studies, insulin release decreased significantly with respect to saline. The percentage reduction in insulin release became statistically significant 60 min after the beginning of the study and achieved values of 44.0 ± 3.8 and 54.6 ± 3.0% during the last 30 min of the equilibration period with low- and high-insulin infusion, respectively ($P < 0.05$ vs. saline; NS between low- and high-insulin infusion).

Arginine test. Glucose concentration increased during the first 30 min of the arginine test to levels of 6.1, 5.5, and 5.5 mmol/l during saline, low-insulin, and high-insulin infusion, respectively ($P < 0.01$ for saline vs. both insulin infusions) and returned to the pretest level within 60 min (Fig. 2*A*). Both plasma insulin and C-peptide responses to arginine were biphasic: the first phase peaked at 3 min, decreasing to a nadir at 10 min and followed by a sustained increase that

TABLE 1
Clinical characteristics of the subjects and C-peptide model parameters obtained using the procedure described by Van Cauter et al. (16)

	Clinical characteristics				Model parameters			
Subject	Type	Age (years)	Body weight (kg)	Height (cm)	k_{12} (min^{-1})	k_{01} (min^{-1})	k_{21} (min^{-1})	V_1 (ml/kg)
1	N	20	64	173	0.0500	0.0606	0.0509	62.8
2	N	25	73	184	0.0497	0.0598	0.0518	60.05
3	N	20	56	165	0.0500	0.0606	0.0509	66.63
4	N	25	78	185	0.0497	0.0598	0.0518	57.68
5	N	19	75	185	0.0502	0.0608	0.0508	59.22
6	N	23	79	190	0.0499	0.0601	0.0515	58.41
7	N	24	79	179	0.0498	0.0599	0.0516	56.22

All subjects are men.

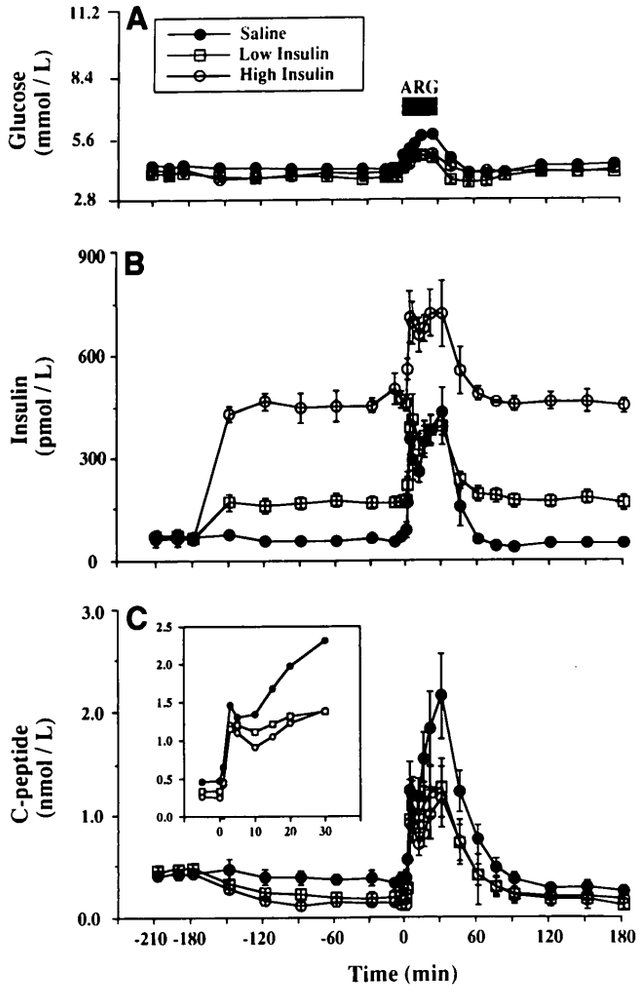

FIG. 2. Glucose (*A*), insulin (*B*), and C-peptide (*C*) levels during saline, low-insulin, and high-insulin infusions in seven subjects (means ± SE). The arginine test (ARG, 25 g in 30 min) started at 0 min.

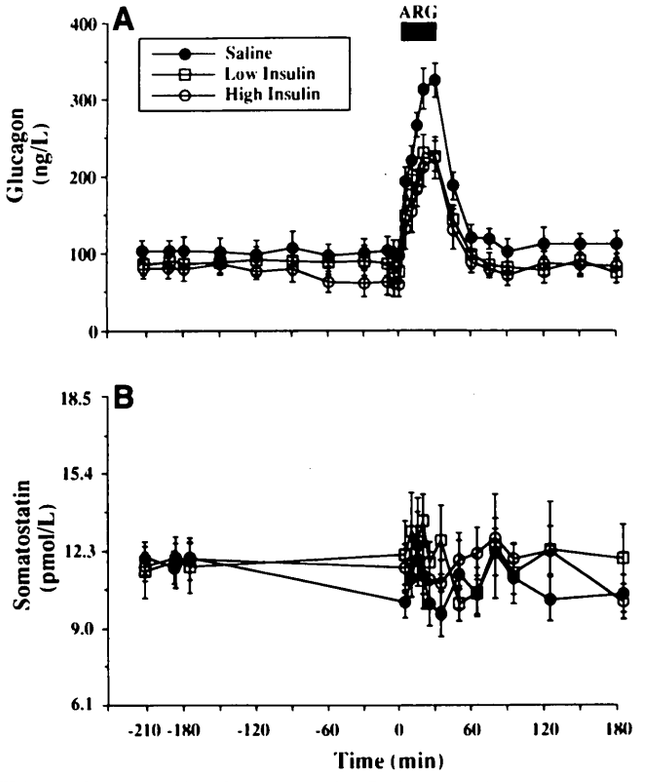

FIG. 3. Glucagon (*A*) and somatostatin (*B*) levels during saline, low-insulin, and high-insulin infusions in seven subjects (means ± SE). The arginine test (ARG, 25 g in 30 min) started at 0 min.

peaked again at 30 min when the arginine infusion was stopped (Fig. 2*B* and *C*). The incremental first- and second-phase AUCs for insulin and C-peptide are reported in Table 3. Glucagon response to arginine was significantly lower during low- and high-insulin infusions than during saline infusion (Table 3). First- and second-phase somatostatin release was completely suppressed during both insulin infusions (Table 3). Plasma FFA, plasma potassium, and plasma calcium levels were not different in the three experiments (Table 2).

Figure 5 shows the three average profiles of insulin release (in staircase fashion) obtained by deconvolution during saline (Fig. 5*A*), low-dose (Fig. 5*B*), and high-dose (Fig. 5*C*) insulin infusion. The estimated insulin responses to infused arginine were biphasic in nature: first-phase release was between 0 and 5 min, and second phase was between 5 and 45 min of the arginine test. From 45 min onward, a refractory period was observed during which insulin release dropped below the pretest level.

Figure 6 displays the magnitude of incremental first- and second-phase insulin response to arginine during saline and the two insulin infusions. The incremental amount of insulin released during the first phase was not different during the saline test and the two insulin infusions. In contrast, the incremental amount of insulin released during the second phase was significantly reduced by 46 and 50% during low- and high-dose insulin infusions, respectively ($P < 0.05$ vs. saline; NS between the two insulin infusions).

TABLE 2
FFA, potassium, and calcium levels during saline and insulin infusions

	Saline			Low insulin			High insulin		
Time (min)	FFA (mmol/l)	Potassium (mEq/l)	Calcium (mEq/l)	FFA (mmol/l)	Potassium (mEq/l)	Calcium (mEq/l)	FFA (mmol/l)	Potassium (mEq/l)	Calcium (mEq/l)
−180	0.55 ± 0.05	4.1 ± 0.1	2.5 ± 0.0	0.53 ± 0.06	4.0 ± 0.1	2.4 ± 0.0	0.53 ± 0.08	4.1 ± 0.1	2.4 ± 0.0
−120	0.55 ± 0.07	—	—	0.51 ± 0.13	—	—	0.39 ± 0.07	—	—
−60	0.66 ± 0.09	—	—	0.56 ± 0.16	—	—	0.41 ± 0.04	—	—
0	0.87 ± 0.17	4.1 ± 0.1	2.5 ± 0.0	0.51 ± 0.21	3.9 ± 0.1	2.4 ± 0.0	0.59 ± 0.14	3.9 ± 0.1	2.5 ± 0.0
+30	0.48 ± 0.12	—	—	0.33 ± 0.07	—	—	0.30 ± 0.08	—	—
+60	0.58 ± 0.18	4.5 ± 0.1	2.4 ± 0.0	0.55 ± 0.14	4.3 ± 0.1	2.4 ± 0.0	0.47 ± 0.09	4.3 ± 0.1	2.4 ± 0.0
+180	1.09 ± 0.16*	4.1 ± 0.1	2.4 ± 0.0	0.39 ± 0.05	4.0 ± 0.1	2.4 ± 0.0	0.44 ± 0.07	4.0 ± 0.1	2.4 ± 0.0

At time 0, 25 g of arginine were infused for 30 min. *$P < 0.05$ vs. the other times during saline and both insulin infusions.

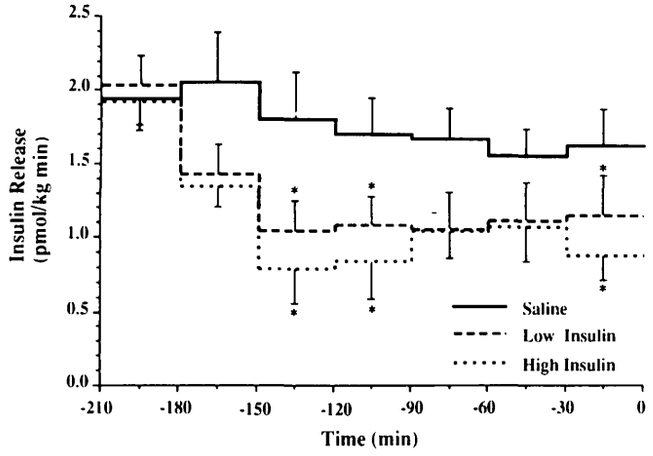

FIG. 4. Time course of insulin release during the 180-min equilibration period in seven subjects (means ± SE). Insulin release during saline, low-insulin, and high-insulin infusions was calculated from C-peptide concentrations by using the two-compartment model of C-peptide kinetics and deconvolution. $P < 0.05$ vs. saline infusion.

DISCUSSION

Our results indicate that hyperinsulinemia reduces the second but not the first phase of arginine-induced insulin release in humans. We found that the amount of insulin released during the first phase of insulin response to arginine was not different during the saline test and during low- and high-dose insulin infusions. In contrast, the amount of insulin released during the second phase of insulin response to arginine was significantly reduced by 46 and 50% during low- and high-dose insulin infusions, respectively. Although many studies have demonstrated the inhibitory effect of insulin on its own secretion (1–6), relatively few studies have examined the effect of hyperinsulinemia on the dynamics of insulin response to different stimuli (7,8). In addition, the inhibition was measured from the AUC of either C-peptide (7) or insulin (8) concentration. In this way, it was not possible to individually assess the first and second phase of insulin release. To our knowledge, this study is the first to quantify the ability of hyperinsulinemia to modulate first- and second-phase insulin response to arginine.

Our results show that the first phase of arginine-induced insulin release is not significantly influenced by hyperinsulinemia. This finding is in apparent contrast with the data reported by Asplin et al. (8). These authors found that the AUC of insulin concentration above the pretest level in the first 10 min after the arginine bolus (an approximate index of first-phase insulin release) fell significantly during hyperinsulinemia in a dose-dependent fashion. The discrepancy between our findings and those of Asplin et al. (8) may be the result of different experimental approaches. In this study, the saline, low-dose, and high-dose insulin studies were performed on different days. By contrast, the effect of increasing hyperinsulinemic levels on insulin release was studied in Asplin et al. (8) using sequential euglycemic clamps with 90-min intervals between each arginine test. We speculate

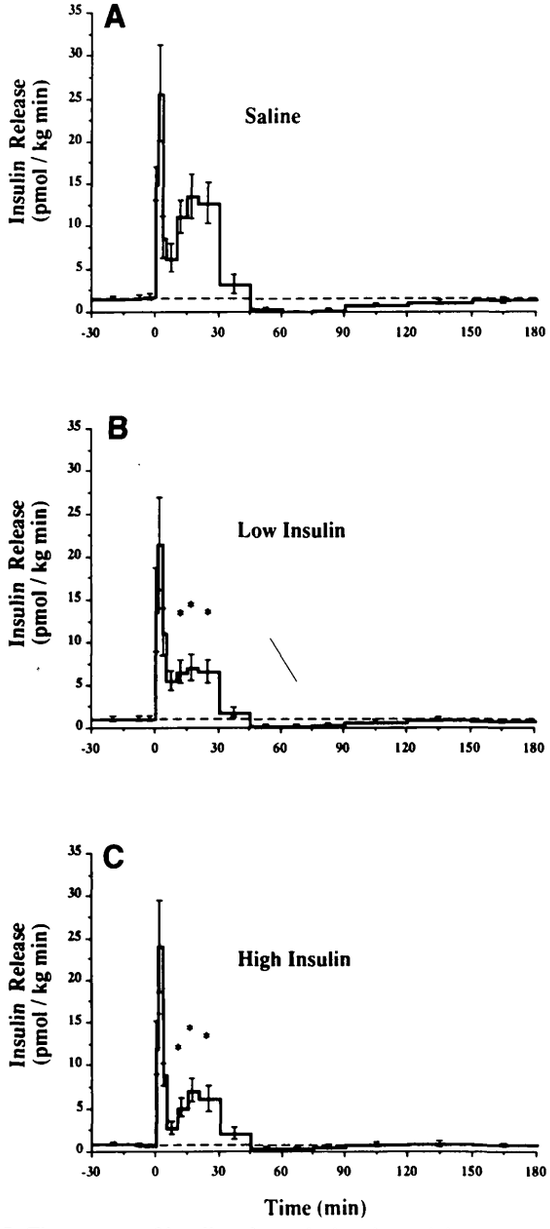

FIG. 5. Time course of insulin release during the arginine test calculated from C-peptide concentrations in seven subjects (means ± SE). Insulin release during saline (*A*), low-insulin (*B*), and high-insulin (*C*) infusions was calculated from C-peptide concentrations by using the two-compartment model of C-peptide kinetics and deconvolution. $P < 0.05$ vs. saline infusion.

TABLE 3
Incremental AUC of pancreatic hormones during the arginine test

	Insulin (0–10 min) (pmol · min^{-1} · l^{-1})	Insulin (10–75 min) (pmol · min^{-1} · l^{-1})	C-peptide (0–10 min) (nmol · min^{-1} · l^{-1})	C-peptide (10–75 min) (nmol · min^{-1} · l^{-1})	IRG (0–90 min) (ng · min^{-1} · l^{-1})	SRIF (0–20 min) (pmol · min^{-1} · l^{-1})	SRIF (20–120 min) (pmol · min^{-1} · l^{-1})
Saline	1,916.4 ± 268.8	9,897.6 ± 1,630.8	7.28 ± 0.86	61.40 ± 10.03	8,632 ± 764	37 ± 13	129 ± 56
Low insulin	1,672.2 ± 295.2	6,274.2 ± 1,655.4*	6.85 ± 1.22	37.17 ± 8.41*	5,738 ± 497*	7 ± 86	−82 ± 15*
High insulin	1,827.0 ± 217.8	7,511.4 ± 1,714.8*	6.32 ± 0.79	39.42 ± 8.84*	6,319 ± 609*	−18 ± 11*	−6 ± 69

IRG, plasma glucagon concentration; SRIF, plasma somatostatin concentration. *$P < 0.05$ vs. saline.

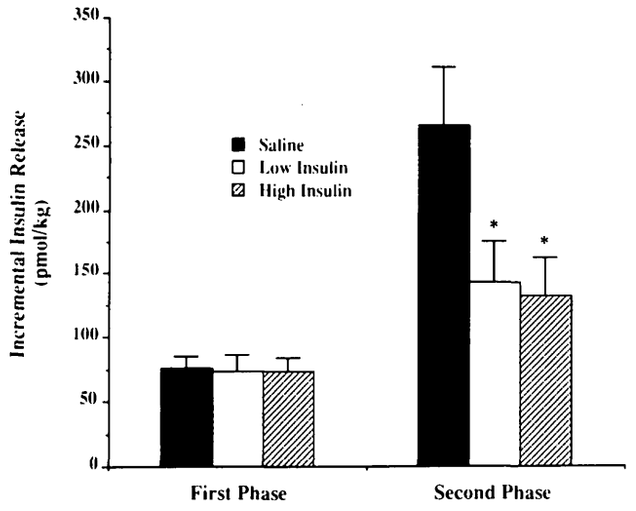

FIG. 6. First- and second-phase insulin release during the arginine test in seven subjects (means ± SE). $P < 0.01$ vs. saline infusion.

that the dose-dependent inhibition of first-phase insulin response observed previously may be a consequence of a too-short time interval between two consecutive arginine stimulations. In fact, Efendic et al. (21) have shown in normal humans that stimulation of the pancreas by arginine causes a refractory state that reduces the insulin response to subsequent stimulations. This memory effect has been shown to be time dependent so that, if enough time is allowed between two consecutive stimulations, the response of the pancreas is not altered. Because we performed the arginine tests on different days, this potentially confounding memory effect is likely to be absent in our study.

Our results show that hyperinsulinemia inhibits second-phase insulin response to arginine. Second-phase insulin release was reduced by 46 and 50% during low- and high-dose insulin studies, respectively. Of note is that the euglycemic clamp technique used did not allow us to correct for differences between the blood glucose responses to arginine during the saline and the two insulin studies, which were probably related to different glucagon responses. This resulted in lower peak glucose values when insulin was infused (5.5 vs. 6.1 mmol/l). To determine whether the lower glucose levels experienced during the insulin studies might have somehow contributed to the observed decrease in second-phase insulin release (arginine exerts its action mainly by potentiating the insulinogenic signal of glucose [13,14,22, 23]), we evaluated the correlation between the difference in the glucose level and the percentage reduction in second-phase insulin release. No correlation was found between these two variables ($r = 0.46$; $P = 0.97$, NS), which indicates that glucose does not play a significant role in the reduction of second-phase insulin response. This result is in agreement with the data reported by Van Haeften et al. (24) that predict (details reported in APPENDIX) that, in our study, glucose accounts for only ~10% of the observed reduction in second-phase insulin response, which leaves the remaining 35–40% to the effect of hyperinsulinemia. To evaluate directly the effect of hyperinsulinemia per se without the confounding effect of blood glucose differences, three additional normal subjects (26 ± 3 years of age; BMI 22 ± 3 kg/m^2) underwent a repeat study in which blood glucose was maintained at similar levels during the arginine test. The subjects under-

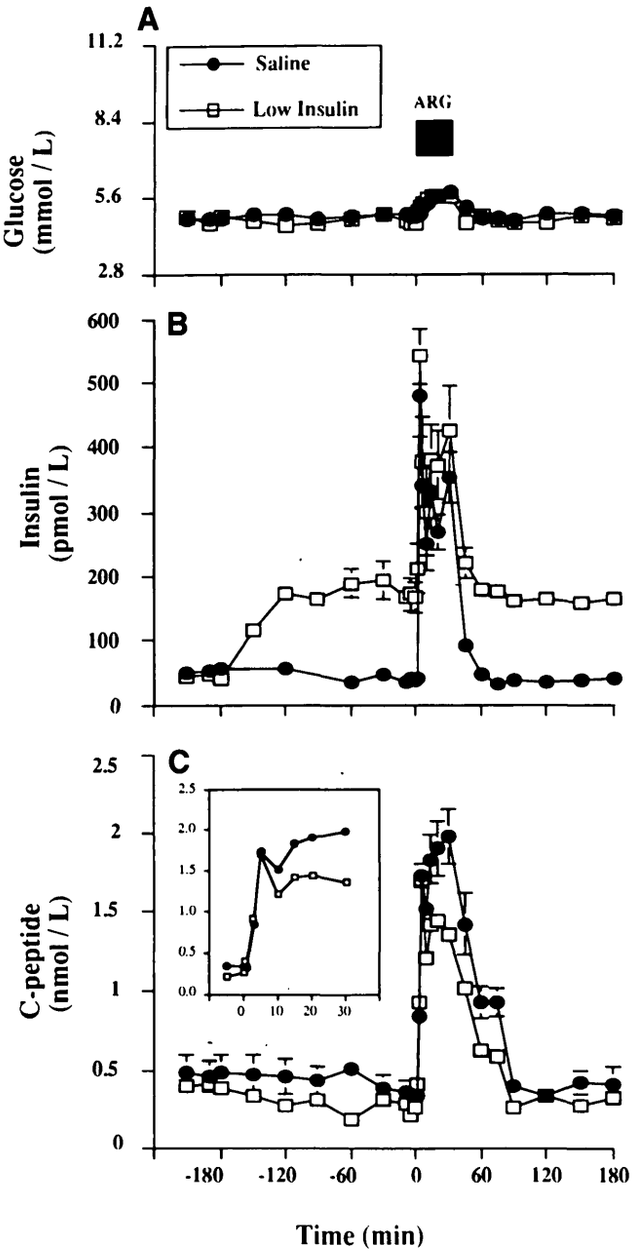

FIG. 7. Glucose (*A*), insulin (*B*), and C-peptide (*C*) levels during saline and low-insulin infusions in three subjects (means ± SE). During low-insulin infusion, blood glucose was clamped at levels achieved during the saline test. In particular, during the arginine test, glucose infusion rate was changed to reproduce similar glucose levels obtained during saline test. The arginine test (ARG, 25 g in 30 min) started at 0 min.

went a control study using saline infusion and a euglycemic clamp using the low-dose insulin infusion. To allow for an accurate matching of glucose levels, the low-dose insulin study was performed after the saline study in each subject. The exogenous glucose infusion rate during the insulin study was varied in such a way as to reproduce the glucose concentration profile measured during the saline experiment. Figure 7 shows the glucose, insulin, and C-peptide levels during the euglycemic period and during the arginine test. Note that glucose levels were virtually superimposable during the two studies, thus allowing for the evaluation of the effect exerted by hyperinsulinemia per se. In addition, during the saline test, blood glucose, insulin, and C-peptide levels were similar to those showed in Fig. 2. Once again we

found that second-phase (271.0 ± 36.3 vs. 176.2 ± 26.7 pmol/kg) but not first-phase (96.2 ± 19.0 vs. 106.9 ± 21.3 pmol/kg) insulin release was reduced during low-insulin infusion. Our data indicate that, in the presence of virtually superimposable glucose levels, the second-phase insulin response to arginine is reduced by 35% during low-insulin infusion. This finding is in close agreement with the result of the theoretical analysis and supports the conclusion that at least 35–39% of the reduction in second-phase insulin release during either low- or high-insulin infusion is due to insulin-modulated inhibition of insulin release.

The results during the 180-min equilibration period also confirm that small, physiological increments in insulin level are able to inhibit endogenous insulin release. An increase in plasma insulin concentration of 120 pmol/l above basal achieved with the low-insulin infusion at the end of the 180-min equilibration period resulted in a 44% decrease in endogenous insulin release. A further increment of 240 pmol/l achieved with the high-insulin infusion induced a further 11% reduction in insulin release, but the difference between the two insulin doses was not significant. Several studies have documented a reduction of insulin release during constant insulin infusion in animals and humans (1–6). However, conflicting evidence exists as to whether the insulin-mediated suppression of insulin release is dose-dependent or not (2,4,5). The observed discrepancies may be due, in part, to different methodological and/or experimental approaches. In this study, we used a methodology similar to the one proposed by Argoud et al. (5), but we did not find the dose-dependent insulin autosuppression found in that study. A possible explanation is that, at variance with Argoud et al.'s study, in our study FFA levels were clamped at the basal level during exogenous insulin administration. Some evidence exists that, in addition to a direct effect of insulin on its own secretion, insulin-induced suppression of lipolysis and plasma FFA concentration may contribute to restrain endogenous insulin release (25,26). Although Argoud et al.'s study (5) reported no FFA data, it stands to reason that an FFA decrement may have occurred. A systematic decrease in FFA levels may have contributed to enhance insulin activity to inhibit its own secretion, thus magnifying the difference between the low-dose and high-dose insulin infusions.

Neither glucagon or somatostatin levels were significantly influenced by either insulin infusion during the equilibration period. During the arginine test, the glucagon and the first-phase somatostatin release were reduced by both insulin infusions, as previously demonstrated by Maruyama et al. (27) and Kazumi et al (28). Because in this study insulin was infused into a peripheral vein, thus reaching the endocrine pancreas through the arterial blood flow, our results substantiate the possibility that insulin affects glucagon and somatostatin release through the vascular stream (29), although we cannot rule out a possible paracrine effect (8).

In conclusion, a quantitative characterization of the feedback effect of hyperinsulinemia on the dynamics of arginine-stimulated insulin release has been accomplished by using C-peptide kinetic modeling and deconvolution. Our results demonstrate that physiological elevations in plasma insulin levels reduce second-phase but not first-phase insulin response to arginine. Furthermore, the inhibitory effect exerted by insulin on second-phase insulin response to arginine is maximized at insulin levels only four times basal.

APPENDIX

The influence of plasma glucose levels on arginine-induced insulin release has been investigated by Van Haeften et al. (24). These authors characterized the dose-response relationship between glycemia and first- and second-phase arginine-induced insulin release. Specifically, the increment in plasma C-peptide level from baseline at 30 min after starting arginine infusion was used as an index of second-phase arginine-stimulated release. Increments in plasma C-peptide levels at this time point were plotted against the prevailing glycemia and were fitted to the modified Michaelis-Menten equation of Grodsky (30):

$$\Delta\text{C-pep}(30) = \frac{V_{\max}\, g^{N}}{(\text{ED}_{50}\text{N} + g^{N})} \qquad \text{(A1)}$$

where ΔC-pep(30) is the measured C-peptide increment at 30 min, V_{max} is the maximal β-cell responsiveness, ED_{50} is the half-maximally stimulating glucose level, N is the exponent added by Grodsky to improve fitting, and g is the ambient glucose level. The dose-response curve of plasma C-peptide increments at 30 min was characterized by the following parameters: V_{max} = 8.5 nmol/l, ED_{50} = 12.7 mmol/l, and N = 2.5. By using this relationship, we calculated that a reduction of 0.6 mmol/l in glucose level from 6.1 to 5.5 mmol/l would correspond to a reduction of 0.17 nmol/l in ΔC-pep(30). To determine the reduction in the second-phase insulin release associated with a reduction of 0.17 nmol/l in the value of ΔC-pep(30), we calculated the relationship between the second-phase insulin release and ΔC-pep(30) in our study. The best regression line (r = 0.90) was

$$\text{second phase} = -6.86 + 144\ \Delta\text{C-pep}(30) \qquad \text{(A2)}$$

with second phase and ΔC-pep(30) expressed in pmol/kg and nmol/l, respectively. Based on this regression line, we calculated that a reduction of 0.17 nmol/l from 1.86 to 1.69 (where 1.86 is the mean value of ΔC-pep(30) during saline infusion) is associated with a 10% reduction in the second phase of insulin release.

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