# Insulin Responses to Nonglucose Stimuli in Non-insulin-dependent Diabetes Mellitus During a Tolbutamide Infusion

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**SUMMARY**

**To determine the effect of tolbutamide on insulin release to nonglucose stimuli in non-insulin-dependent diabetes mellitus and how plasma glucose levels may modulate this effect, the acute insulin response (AIR) to an isoproterenol (12 $\mu$g) or an arginine (5 g) i.v. pulse was determined before and during a tolbutamide infusion (7.5 mg/m$^2$/min) in 25 male subjects. During the tolbutamide infusion, there was an increase in the AIR to both isoproterenol (% $\Delta$ AIR = +49 ± 21%, N = 11, $P < 0.05$) and arginine (% $\Delta$ AIR = +52 ± 15%, N = 12, $P < 0.005$) and a decrease in plasma glucose ($\Delta$ plasma glucose for isoproterenol = −24 ± 6 mg/dl, $P < 0.005$; for arginine = −26 ± 3 mg/dl, $P < 0.001$). In separate studies, when the plasma glucose was clamped at baseline values by a variable rate of glucose infusion, there was a greater effect of tolbutamide on AIR when compared with the unclamped tolbutamide studies (isoproterenol: % $\Delta$ AIR = +132 ± 25%, $P < 0.025$; arginine: % $\Delta$ AIR = +95 ± 12%, $P < 0.05$). Thus, tolbutamide increases the AIR of nonglucose stimuli, but this augmentation by tolbutamide is blunted by the concomitant decrease in plasma glucose. Consideration of this observation is necessary when interpretating the effects of a sulfonylurea on islet cell responses.**

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It has been demonstrated that insulin secretion in response to nonglucose stimuli is dependent on the prestimulus glucose level in both normal and diabetic subjects.[1–4] These nonglucose stimuli include a beta-adrenergic agonist (isoproterenol[1]), an amino acid (arginine[2]), and several gut hormones (GIP[3] and secretin[4]). The potentiation by glucose of insulin secretory responses to these stimuli is such that the higher the plasma glucose level at the time of the stimulus (prestimulus glucose level), the greater the insulin secretion; and the lower the prestimulus glucose level, the smaller the insulin secretory response to the stimulus. This phenomenon has been termed glucose potentiation. We have recently shown that insulin secretory responses of normal subjects are also dependent on the prestimulus glucose level during the administration of the drug tolbutamide.[5] In these normal subjects, the decreased plasma glucose levels that accompanied the administration of tolbutamide tended to mask the insulinotropic effects of this drug. It was not until the plasma glucose levels were prevented from falling by a concomitant glucose infusion during the administration of this sulfonylurea that the insulinotrophic effects became apparent. The present study was designed to determine if sulfonylureas can enhance insulin secretion in response to nonglucose stimuli in diabetic subjects and to determine the relationship of these effects to changes of prestimulus glucose levels accompanying tolbutamide administration.

## MATERIALS AND METHODS

**Subjects.** Twenty-five non-insulin-dependent male diabetic subjects were studied. These subjects tended to be overweight, with an average percent ideal body weight of 128 ± 4% ($\bar{x}$ ± SEM; range: 84–168%) as assessed from the Metropolitan Life Insurance Tables, 1959. The average age was 56 ± 2 yr, with a range of 37–71 yr. None of the subjects were on any medications, and none had a history of cardiovascular disease. Diabetes mellitus was recently diagnosed in all subjects, with an average duration of disease of 2 ± 0.5 mo (range: 1–7 mo). All subjects had a fasting plasma glucose of 115 mg/dl or greater,[6] with an average fasting plasma glucose of 196 ± 16 mg/dl (range: 115–376 mg/dl).

**Study protocol.** All subjects were studied after an overnight fast, and no cigarette smoking was allowed on the day of the study. Aspirin and aspirin-containing products were not allowed for a period of at least 1 wk before the studies.[7] The

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subjects were on an ad libitum diet. Informed consent was obtained before any studies were performed. All studies were performed in a metabolic ward. Butterfly needles (19-gauge) were introduced into both antecubital veins, and each was kept patent by a slow infusion of 0.9% sodium chloride. After the line had been cleared of saline by withdrawing and discarding 1.5 ml of blood, venous samples were obtained from one line for laboratory analysis. The other intravenous line was used for administration of the drugs used during the study.

The beta-adrenergic agonist, isoproterenol, and the amino acid, arginine, were used as the nonglucose insulin secretagogues. Isoproterenol was given rapidly (in less than 3 s) at a dose of 12 $\mu$g, which has been determined to be a maximal stimulus for acute insulin release in man.[8] The acute insulin response (AIR) to isoproterenol was calculated as the mean increase above the prestimulus plasma insulin levels at 2, 3, and 4 min after the isoproterenol pulse.[8] In other studies, 5 g of L-arginine monohydrochloride solution (10%) was given rapidly (in less than 15 s) intravenously. This dose has also been determined to be a maximal stimulus for insulin secretion in man.[9] The AIR to arginine was calculated as the mean increase above prestimulus plasma insulin levels at 2, 3, 4, and 5 min after the administration of the amino acid.[5]

In all studies, a 45-min basal period was followed by a pulse of isoproterenol or arginine. Venous samples were taken rapidly during the first 10 min after the pulse and then less frequently for an additional 20 min. Thirty minutes after the first secretagogue pulse, an infusion of tolbutamide was begun at a rate of 7.5 mg/m²/min. This dose was chosen because, in normal man, it resulted in a 20% fall in venous glucose levels over a 1-h period without stimulating an increase in plasma catecholamine levels.[10] After 1 h of this infusion, a second pulse of either isoproterenol or arginine was given. Previous studies have shown that the insulin response to a pulse of isoproterenol or arginine is not affected by a previous pulse of that secretagogue.[5,9] The AIR to the second isoproterenol or arginine pulse was expressed both in absolute terms as well as a percentage of the AIR of the first pulse.

In separate studies, a variable glucose infusion was concomitantly administered during the tolbutamide infusion to prevent a change of plasma glucose level (glucose clamp). The glucose infusion was begun at 100 or 200 mg/min, 5 min after the beginning of the tolbutamide infusion. The infusion rate was subsequently adjusted at 5-min intervals depending on the results of bedside plasma glucose measurements with the Beckman glucose analyzer (Beckman Instruments, Fullerton, California). These results were available within 2 min of the time of sampling. An empirically derived formula[1] was used for calculating the glucose infusion rate. This variable glucose infusion was continued for the 55 min before the second secretagogue pulse and then was maintained at the last rate before the second pulse for the duration of the study.

Five-milliliter samples for measurement of plasma immunoreactive insulin (IRI) and glucose were anticoagulated by ethylenediaminetetraacetic acid (EDTA), kept on ice until the plasma was separated by centrifugation at 4°C, and subsequently frozen at −20°C for analysis at a later time. Two-and-one-half-milliliter samples for measurement of plasma catecholamines (norepinephrine and epinephrine) were obtained 5 min before and immediately preceding all secretagogue pulses to ensure that the decrease in plasma glucose did not elicit an adrenergic response. The mean of these two measurements was used for data analysis. These samples were collected in chilled tubes containing ethyleneglycoltetraacetic acid (EGTA) for anticoagulation and glutathione to prevent oxidation, kept on ice until the plasma was separated by a double centrifugation at 4°C (within 30 min), and subsequently frozen at −20°C for analysis at a later time. Ten-milliliter samples for measurement of serum tolbutamide levels were obtained immediately preceding the second pulse. These samples were allowed to clot. The serum was separated from the clot and frozen at −20°C for analysis at a later time.

**Analytical methods.** Plasma IRI levels were measured by a modification of the double antibody method of Morgan and Lazarow.[11] Plasma glucose was measured with the AutoAnalyzer glucose oxidase method (Technicon Instruments). Bedside plasma glucose was determined by a glucose oxidase method (Beckman Instruments), but these measurements were not used for data analysis. Plasma norepinephrine and epinephrine were measured by the single isotope enzymatic assay.[12] Serum tolbutamide measurements were made by the Upjohn Company by a gas-liquid chromatographic method.[13] Statistical analyses included paired and nonpaired Student's *t* test.

## RESULTS

### TOLBUTAMIDE ALONE PROTOCOL

**Responses to isoproterenol.** In 11 non-insulin-dependent diabetic subjects, 12 $\mu$g pulses of isoproterenol were given before and during a 7.5 mg/m²/min tolbutamide infusion (Figure 1). This infusion of tolbutamide resulted in a decrease in plasma glucose ($\Delta$ plasma glucose: $-24 \pm 6$ mg/dl, $P < 0.005$). However, this fall of plasma glucose did not result in an increase of plasma catecholamines (Table 1). During the tolbutamide infusion, plasma insulin levels increased ($\Delta$ plasma IRI = $+12 \pm 2$ $\mu$U/ml, $P < 0.001$) to a new plateau by 15 min and were maintained at this level until the second isoproterenol pulse. The AIR to isoproterenol was greater during the tolbutamide infusion than the response before the tolbutamide ($46 \pm 9$ vs. $34 \pm 7$ $\mu$U/ml, $P < 0.02$), an increase of $49 \pm 21\%$ ($P < 0.05$, Table 1).

**Responses to arginine.** In 12 non-insulin-dependent diabetic subjects, 5 g of arginine was given before and during the tolbutamide infusion (Figure 2). During the tolbutamide there was a significant fall in plasma glucose ($\Delta$ plasma glucose: $-26 \pm 3$ mg/dl, $P < 0.001$). This did not result in an increase in adrenergic activity as assessed by plasma catecholamines (Table 1). The plasma insulin levels increased during the tolbutamide infusion ($\Delta$ plasma insulin: $+14 \pm 2$ $\mu$U/ml, $P < 0.001$). The AIR to arginine was also greater during the tolbutamide infusion ($40 \pm 8$ vs. $60 \pm 17$ $\mu$U/ml, $P < 0.05$), an increase of $52 \pm 15\%$ ($P < 0.005$, Table 1).

### TOLBUTAMIDE-PLUS-GLUCOSE CLAMP PROTOCOL

**Responses to isoproterenol.** In six non-insulin-dependent diabetic subjects, the tolbutamide was accompanied by a variable concomitant glucose infusion to maintain glucose

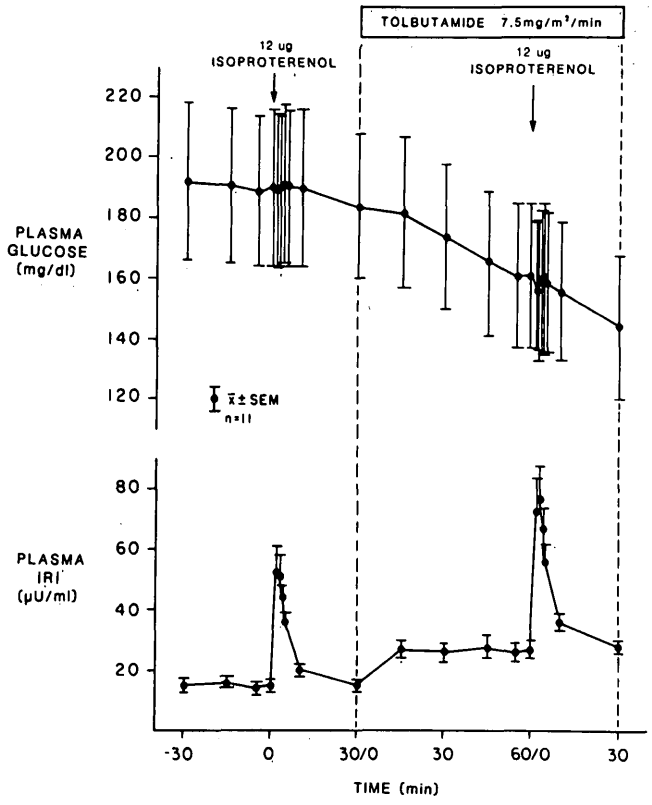


**FIGURE 1. Insulin and glucose responses to 12 μg of isoproterenol before and during a tolbutamide infusion. During the tolbutamide infusion, there was a significant decrease in plasma glucose ($P < 0.005$), and the acute insulin response (x̄ Δ 2–4′ IRI) to isoproterenol increased by 49 ± 21% ($P < 0.05$).**

levels at basal values (Figure 3). During this clamped study, (Δ plasma glucose = +2 ± 3 mg/dl, P = NS), there was a gradual increase in plasma insulin (Δ plasma IRI = +30 ± 5 μU/ml, $P < 0.005$). The AIR to isoproterenol during the tolbutamide-plus-glucose-clamp infusion was greater than baseline AIR (117 ± 19 vs. 54 ± 10 μU/ml, $P < 0.001$, Table 1), an increase of 132 ± 25% above the baseline AIR ($P < 0.001$).

Both the absolute increase in the AIR (+63 ± 9 vs. +12 ± 4 μU/ml, $P < 0.001$) and the percent increase above baseline AIR (+132 ± 25 vs. +49 ± 21%, $P < 0.025$) was greater during the tolbutamide-plus-glucose clamp protocol than the tolbutamide alone protocol. This increase in AIR could not be accounted for by an increase in tolbutamide level (7.88 ± 0.33 vs. 8.12 ± 0.62 mg/dl, P = NS) nor by a difference of plasma catecholamines (Table 1).

**Responses to arginine.** In seven of these diabetic subjects, 5-g arginine pulses were given before and during the tolbutamide-plus-glucose clamp protocol. During this protocol, there was no significant change in the plasma glucose level (Δ plasma glucose: +3 ± 4 mg/dl, P = NS, Figure 4), and there was an increase in the plasma insulin concentration (Δ plasma insulin: +26 ± 6 μU/ml, $P < 0.005$). There was also an increase in the AIR to arginine (98 ± 29 vs. 53 ± 19 μU/ml, $P < 0.01$), an increase of 95 ± 12% ($P < 0.001$, Table 1).

The AIR to arginine during the tolbutamide-plus-glucose-clamp infusion was greater than that during tolbutamide alone (+46 ± 11 vs. +21 ± 9 μU/ml), but this difference was of borderline significance ($0.05 < P < 0.10$). The percent increase of AIR to arginine was also greater during the

TABLE 1
Effects of a tolbutamide infusion on glucose and insulin responses to isoproterenol and arginine

| | Prestimulus | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Glucose (mg/dl) | IRI (μU/ml) | NE (pg/ml) | EPI (pg/ml) | Tolb (mg/dl) | AIR* (μU/ml) | ΔAIR† (μU/ml) | %ΔAIR‡ |
| Tolbutamide Protocol | | | | | | | | |
| Isoproterenol (N = 11) | | | | | | | | |
| Baseline | 191 ± 26 | 15 ± 2 | 245 ± 20 | 52 ± 8 | — | 34 ± 7 | | |
| Tolb INF | 161 ± 25‖ | 27 ± 3¶ | 222 ± 19 | 48 ± 8 | 8.12 ± 0.62 | 46 ± 9§ | +12 ± 4 | +49 ± 21 |
| Arginine (N = 12) | | | | | | | | |
| Baseline | 214 ± 27 | 16 ± 1 | 255 ± 18 | 54 ± 10 | — | 40 ± 8 | | |
| Tolb INF | 188 ± 26¶ | 30 ± 3¶ | 245 ± 16 | 49 ± 7 | 7.68 ± 0.51 | 60 ± 17§ | +21 ± 9 | +52 ± 15 |
| Tolbutamide + glucose protocol | | | | | | | | |
| Isoproterenol (N = 6) | | | | | | | | |
| Baseline | 135 ± 8 | 24 ± 4 | 272 ± 76 | 56 ± 10 | — | 54 ± 10 | | |
| Tolb + glucose clamp | 137 ± 11 | 54 ± 6‖ | 280 ± 84 | 61 ± 11 | 7.85 ± 0.33 | 117 ± 19¶ | +63 ± 9** | +132 ± 25# |
| Arginine (N = 7) | | | | | | | | |
| Baseline | 205 ± 26 | 20 ± 5 | 220 ± 51 | 39 ± 10 | — | 53 ± 19 | | |
| Tolb + glucose clamp | 209 ± 26 | 47 ± 8‖ | 202 ± 37 | 39 ± 10 | 7.51 ± 0.25 | 98 ± 29‖ | +46 ± 11 | +96 ± 12# |

All values are x̄ ± SEM. IRI = immunoreactive insulin; NE = norepinephrine; EPI = epinephrine; Tolb = tolbutamide; AIR = acute insulin response; Tolb INF = tolbutamide infusion; Tolb + clamp = tolbutamide during glucose clamp.
* AIR = x̄ Δ 2–4′ IRI for isoproterenol; AIR = x̄ Δ 2–5′ IRI for arginine.
† For each subject the ΔAIR was calculated: ΔAIR = AIR during tolbutamide minus baseline AIR; values shown in the table are x̄ ± SEM for each group.
‡ For each subject the %ΔAIR was calculated: %ΔAIR = [(AIR during tolbutamide minus baseline AIR) ÷ (baseline AIR)] × [100]; values shown in the table are x̄ ± SEM for each group.
§ $P < 0.05$; ‖ $P < 0.01$; ¶ $P < 0.001$, difference from baseline (paired *t* test).
# $P < 0.05$; ** $P < 0.001$, difference between Tolb + clamp vs. Tolb INF (nonpaired *t* test).

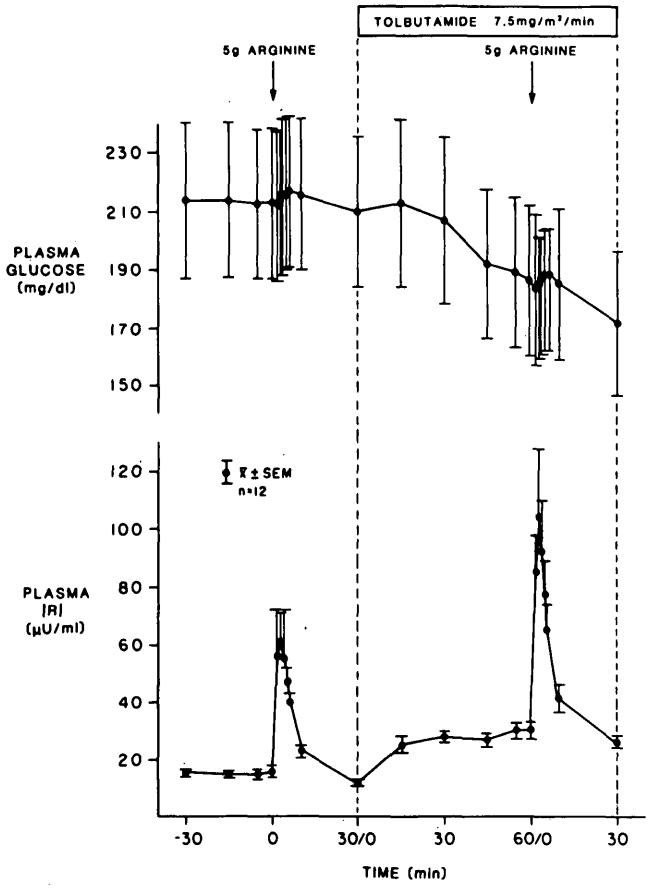


**FIGURE 2. Insulin and glucose responses to 5 g of I.v. arginine before and during a tolbutamide infusion. During the tolbutamide infusion, there was a decrease in plasma glucose ($P < 0.001$), and the acute insulin response (x̄ Δ 2–5′ IRI) to arginine increased by 52 ± 15% ($P < 0.005$).**

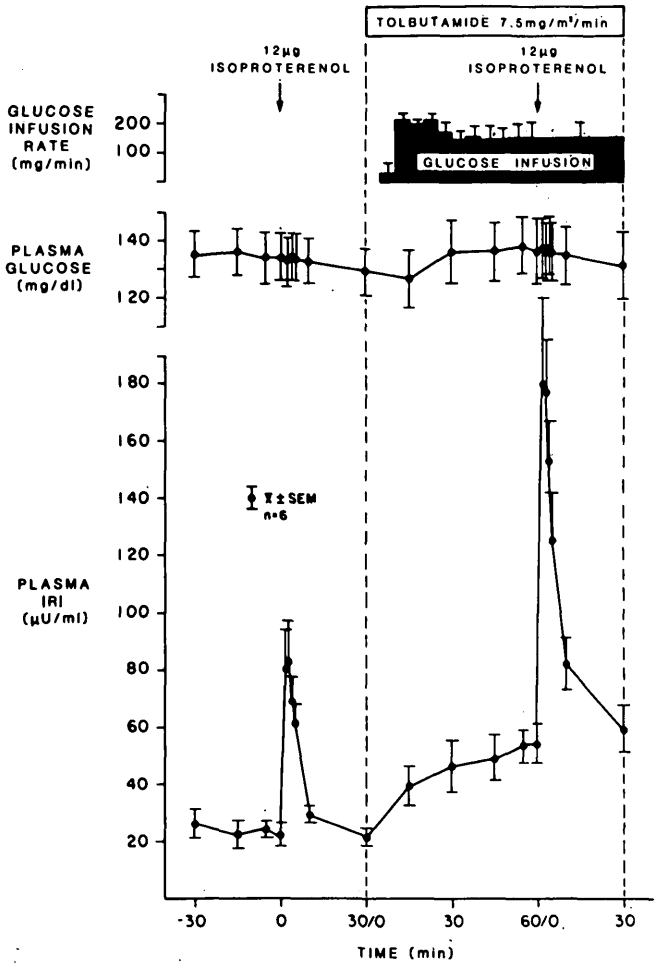


**FIGURE 3. Insulin and glucose responses to 12 μg of isoproterenol before and during tolbutamide infusion with glucose clamped. During the glucose-clamped-tolbutamide infusion, the acute insulin response to 12 μg of isoproterenol increased by 132 ± 25% ($P < 0.001$), a greater increase ($P < 0.05$) than that observed during tolbutamide alone (see Figure 1).**

clamp study (+95 ± 12 vs. +52 ± 15%, $P < 0.05$). This increase in AIR could not be accounted for by an increase in tolbutamide levels nor by a difference change in plasma catecholamines (Table 1).

## DISCUSSION

These studies demonstrate that the infusion of the sulfonylurea, tolbutamide, results in an increase in the acute insulin response to the nonglucose stimuli, isoproterenol and arginine, in patients with non-insulin-dependent diabetes mellitus (NIDDM). However, the effect of tolbutamide was partially masked by the concomitant decrease of plasma glucose during the tolbutamide infusions. When the plasma glucose concentration was prevented from falling by a concomitant glucose infusion, there was a greater augmentation of the AIR to both isoproterenol and arginine (Table 1). The glucose level dependency on the effect of tolbutamide in patients with NIDDM is similar to those we observed in normal subjects during an infusion of tolbutamide.[5] In normal subjects, the fall in plasma glucose completely masked the insulinotrophic effects of tolbutamide; only when the plasma glucose was clamped at baseline values could the augmented insulin secretion during the tolbutamide infusion be readily demonstrated. These findings indicate that the prestimulus plasma glucose concentration is an important determinant of islet cell responsiveness to nonglucose stimuli during tolbutamide both in normals and diabetics.

Since diabetics are less sensitive than normals to the potentiating effect of glucose on insulin responses to nonglucose stimuli,[1] it would presumably take a larger change of plasma glucose to completely mask the tolbutamide effect. Thus, although the same decline in glucose level was achieved, small but significant increases of insulin responses to both isoproterenol and arginine were still observed in the diabetic subjects during the tolbutamide infusion even when the glucose level was not clamped (Table 1).

We have previously shown that in a cross-sectional analysis of normal and untreated non-insulin-dependent diabetic subjects, there is no simple relationship between the absolute plasma glucose level and the AIR to nonglucose stimuli.[14] However, for a given individual, changes in prestimulus plasma glucose level will result in changes in the AIR to nonglucose stimuli.[1] This relation is such that a decrease in prestimulus plasma glucose level will result in a smaller AIR and an increase will result in a larger AIR. Therefore, interpretation of these and similar studies are

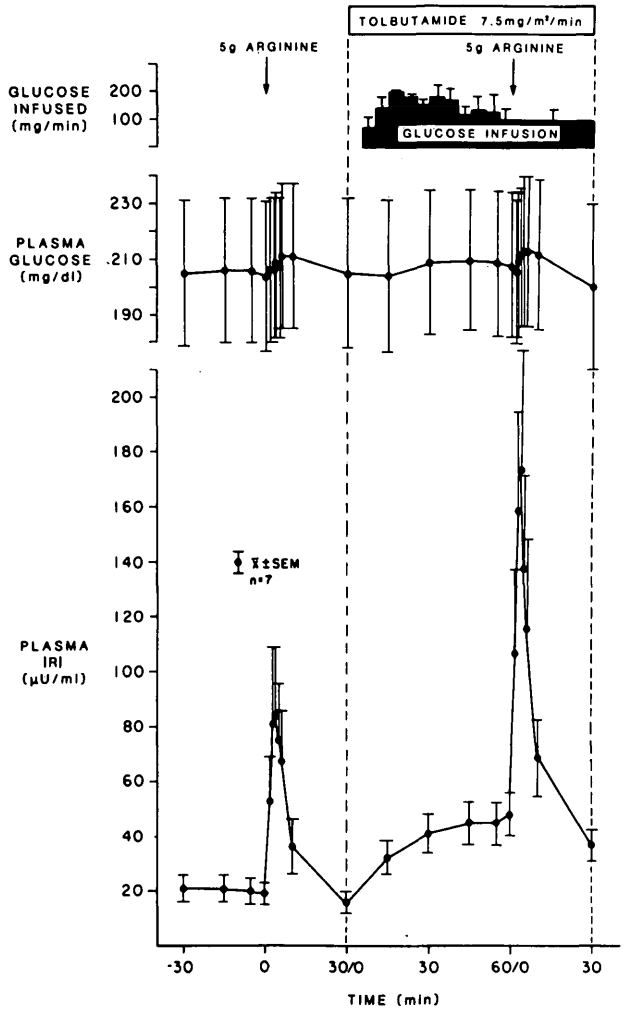


**FIGURE 4. Insulin and glucose responses to 5 g of i.v. arginine before and during a tolbutamide-plus-glucose-clamp infusion. The acute insulin response during the infusion period was increased by 95 ± 12% ($P < 0.01$), a greater increase ($P < 0.05$) than that during tolbutamide alone (see Figure 2).**

the prestimulus plasma glucose level to the individual fasting plasma glucose level.

Previous studies of islet effects of sulfonylureas in diabetics have led to some confusion. During therapy of diabetes mellitus with a sulfonylurea drug, it has been reported that insulin secretion increases shortly (days) after the initiation of sulfonylurea therapy,[15–20] but that during longer (months) therapy insulin secretion appears to be unchanged from pretreatment values.[15–18,21–23] These findings have been interpreted as evidence that the insulinotrophic effects of sulfonyureas are short lived. However, our findings demonstrate that the prestimulus plasma glucose level must be considered when evaluating the effects of tolbutamide on insulin secretory responses of patients with NIDDM. Thus, it is likely that a decrease in plasma glucose during chronic sulfonylurea therapy may mask the insulinotrophic effects of the drug.

In summary, tolbutamide infusion results in increased insulin responses to isoproterenol and arginine in patients with NIDDM. However, this effect of the drug is muted by the concurrent decrease in plasma glucose concentration. Thus, the tolbutamide infusion resulted in even greater insulin responses when the plasma glucose was clamped at baseline levels. We hypothesize that changes of plasma glucose during chronic sulfonylurea therapy may similarly tend to reduce and may even obscure important effects of these drugs on islet function. Studies of insulin secretory responses in diabetics during chronic sulfonylurea therapy will be required to test this hypothesis.

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