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Antecedent hypoglycemia does not alter increased epinephrine-induced lipolysis in type 1 diabetes mellitus

Robert P. Hoffman*

Division of Pediatric Endocrinology, Department of Pediatrics, The Ohio State University College of Medicine and Public Health, Columbus, OH 43205, USA
The Clinical Research Center, The Ohio State University College of Medicine and Public Health, Columbus, OH 43210, USA
The Children's Research Institute, Columbus, OH 43205, USA

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Abstract

Type 1 diabetic subjects have decreased epinephrine responses to hypoglycemia that may be counterbalanced by increased β-adrenergic sensitivity. The goal of this study was to determine whether type 1 diabetic subjects have increased metabolic response to epinephrine and to determine the effect of antecedent hypoglycemia on these responses. Muscular glucose uptake across the forearm (forearm glucose uptake, Fick principle) and lipolysis (free fatty acid and glycerol levels) were studied before and during a 4-hour euglycemic, hyperinsulinemic (40 mU $\cdot$ m^{-2} $\cdot$ min^{-1}) clamp with epinephrine infusion (0.015 $\mu g \cdot kg^{-1} \cdot min^{-1}$) over 3 hours. Subjects were studied twice, once with antecedent hypoglycemia (2.8 mmol/L for two 2-hour sessions) and once with antecedent euglycemia (5 mmol/L) the day prior. Free fatty acid and glycerol concentrations were higher, and total body glucose utilization and forearm glucose uptake during epinephrine were lower in diabetic than in control subjects ($P < .05$). Antecedent hypoglycemia had no effect. These results demonstrate that type 1 diabetic subjects have increased lipolysis and decreased glucose utilization in response to epinephrine. These effects are not altered by antecedent hypoglycemia.

1. Introduction

Hypoglycemia is the limiting factor in achieving near-normal glycemia in patients with type 1 diabetes mellitus. Unfortunately, in addition to being unable to regulate exogenous insulin once given, these patients also have absent glucagon secretion in response to hypoglycemia and many have decreased epinephrine secretion [1-4]. The decreased epinephrine response is likely due to recurrent hypoglycemia [5,6] because hypoglycemia on the day prior has been demonstrated to reduce the epinephrine response to insulin-induced hypoglycemia the following morning in control and type 1 diabetic individuals [5-7].

It has also been suggested, however, that alterations in β-adrenergic responsiveness may affect glucose counter-regulation in type 1 diabetic patients. Patients with type 1 diabetes and hypoglycemic unawareness have been shown to have diminished heart rate responses to isoproterenol [8]. This finding of diminished cardiac β-adrenergic sensitivity in patients with type 1 diabetes mellitus has been confirmed in at least 2 subsequent studies [9,10]. A single episode of nocturnal hypoglycemia has been shown to increase β-adrenergic sensitivity in healthy subjects and decrease β-adrenergic sensitivity in type 1 diabetic subjects [11]. From 2 case reports (1 diabetic patient [12], 1 patient with insulinoma [13]), it appears that avoidance of hypoglycemia can lead to restoration of normal β-adrenergic sensitivity.

Cardiac β-adrenergic sensitivity however has little to with epinephrine's counterregulatory effects, and it is unclear whether the decreased cardiac β-adrenergic sensitivity in type 1 diabetes mellitus extends to its metabolic effects because adrenergic sensitivity may differ between tissues [14]. Epinephrine aids recovery from hypoglycemia by 3 major mechanisms: increasing endogenous glucose production [15,16], decreasing peripheral insulin sensitivity [17,18], and increasing lipolysis to generate alternate fuel [19-21]. The first goal of this study was to determine whether the reported differences in cardiac β-adrenergic sensitivity between type 1 diabetic and control subjects

* Children's Hospital ED541, Columbus, OH 43205, USA. Tel.: +1 614 722 4425; fax: +1 614 722 4440.
E-mail address: hoffmanr@pediatrics.ohio-state.edu.

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apply to the metabolic responses. The second was to determine the effect of antecedent hypoglycemia on the metabolic responses to epinephrine in control and type 1 diabetic subjects.

2. Subjects, materials, and methods

2.1. Subjects

Eleven subjects (9 males, 2 females) with type 1 diabetes mellitus and 7 control subjects (6 males, 1 female) were studied. Mean age was 25.1 ± 6.9 years (mean ± SD) for the type 1 diabetic subjects and 25.6 ± 3.3 years for control subjects. Mean body mass indices were 24.4 ± 3.6 and 26.1 ± 3.9 kg m^{-2}, respectively. Mean duration of diabetes was 8.7 ± 2.0 years. Mean HbA_{1c} for the diabetic patients was 8.7% ± 2.0% (range, 6.1%-12.3%). Diabetes onset was before 30 years of age, and all had received only insulin therapy from the time of diagnosis. Subjects were free from diabetic complications as determined by history and physical examination. All had normal sensation to light touch. A spot urine sample was collected for measurement of microalbuminuria, and subjects with levels of more than 20 μg microalbumin per milligram of creatinine were excluded. Subjects on medications other than insulin, l-thyroxine replacement, or oral contraceptives in female subjects were excluded. The 1 subject on l-thyroxine replacement had normal thyroid function tests. Subjects who had had a hypoglycemic reaction requiring assistance within the last 3 months were also excluded. Control subjects met similar criteria and were on no medications. Informed consent was obtained from all subjects and the study was approved by the Ohio State University Office of Responsible Research Institutional Review Board.

2.2. Protocol

Both control and type 1 diabetic subjects were admitted to the Clinical Research Center the night before study. Diabetic subjects received their usual evening intermediate (isophane, lente), short (regular), or rapid-acting (lispro) or long-acting (ultralente, glargine) insulin. Patients on continuous subcutaneous insulin infusion received their basal insulin overnight. Blood glucose levels were checked overnight by means of a blood glucose monitor at midnight and at 3:00 AM, and subjects with a level of less than 3.6 mmol/L were not studied the next day (Fig. 1).

At 8 AM of day 1 of the study, a 2-hour euglycemic or hypoglycemic insulin clamp was initiated (40 mU · m^{-2} · min^{-1}) with arterialized plasma glucose maintained at either 5 or 2.8 mmol/L, as randomly determined. After 2 hours the insulin infusion was stopped, and the plasma glucose concentration was allowed to increase to euglycemic levels, if necessary. In the diabetic subjects, a low-dose insulin infusion was used as needed to maintain the plasma glucose concentration between 4.4 and 6.7 mmol/L. At noon, a second insulin clamp was performed. Both clamps on day 1 were either euglycemic or hypoglycemic [22]. After the second clamp, all subjects were given a meal, and glucose levels were monitored overnight. A variable, low-dose insulin infusion was given to the diabetic subjects to avoid overnight hypoglycemia and to attempt to maintain euglycemia. Blood samples were drawn before starting the first clamp and at the end of

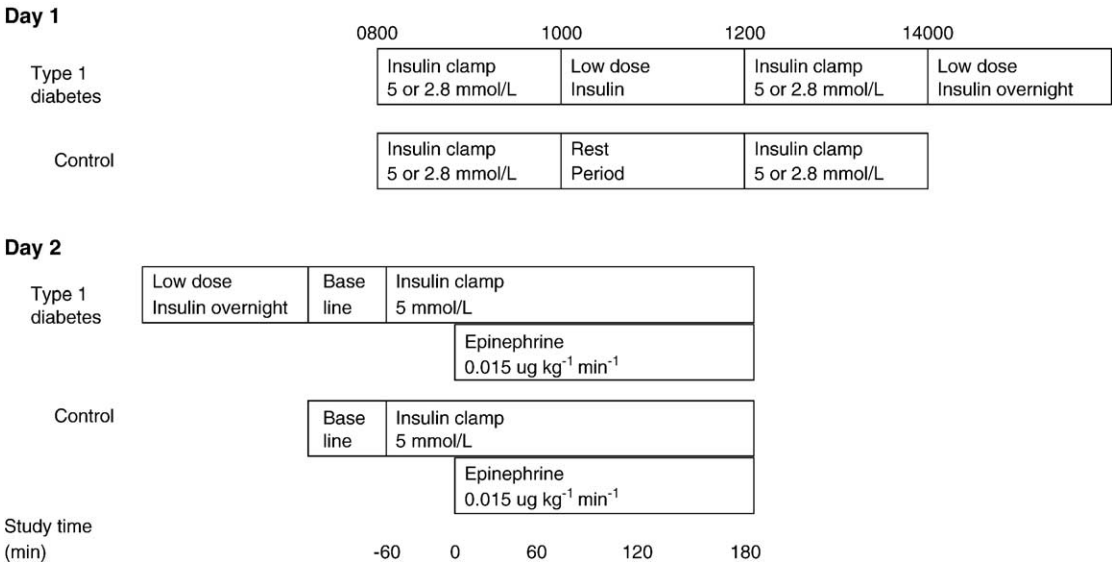

Fig. 1. Protocol for study of control and type 1 diabetic subjects. Subjects were studied with either day 1 hypoglycemia (glucose held at 2.8 mmol/L on day 1 for both clamps) or euglycemia (glucose held at 5 mmol/L on day 1 for both clamps). Low-dose insulin infusion was used only in type 1 diabetic subjects. Subjects were allowed to eat after completion of both clamps on day 1.

each clamp for measurement of venous epinephrine, glucagon, and cortisol concentrations.

On day 2, the main study took place. A deep intravenous catheter was placed in the antecubital fossa of the dominant arm to be used for venous blood sampling, and a radial artery line was placed in the nondominant arm for arterial blood sampling and blood pressure monitoring [23]. Baseline data were collected over 30 minutes without insulin in the control subjects, and then an insulin infusion (40 mU · m^{-2} · min^{-1}) was begun. Arterial and venous blood samples were drawn every 5 minutes and immediately analyzed for plasma glucose concentration. In type 1 diabetic subjects, arterial plasma glucose levels were allowed to fall to 5 mmol/L, and then a 20% dextrose infusion was started to maintain this level.

After 1 hour of insulin infusion, an epinephrine infusion was started at a rate of 0.015 μg · kg^{-1} · min^{-1} [24]. This dose has been shown to lead to plasma epinephrine concentrations in the physiological range similar to those found during hypoglycemia.

Forearm blood flow (FBF) was measured, as described below for 5 minutes out of every 10 minutes. Blood was drawn every 30 minutes for measurement of venous plasma glycerol, insulin, and nonesterified fatty acid (NEFA) levels. Samples were drawn before starting insulin, before starting epinephrine infusion, and at the end of the study for measurement of venous epinephrine, glucagon, and cortisol concentrations.

Subjects returned for a second study with either antecedent euglycemia or hypoglycemia depending on which was previously performed. The 2 studies were conducted at least 2 weeks apart. Two diabetic subjects participated in only the antecedent hypoglycemia study, and 2 diabetic subjects participated in only the antecedent euglycemia study.

2.3. Calculations

Forearm glucose uptake (FGU) was calculated using the Fick principle: FGU = [arterial glucose − venous glucose (arterial-venous glucose difference, or AVDIFF)] × FBF. Total body glucose utilization (M) was calculated using the glucose infusion rate divided by body weight minus space correction factor [0.095 × (glucose difference over time)] [25].

2.4. Measures

Two sphygmomanometric cuffs and an indium-in-silastic strain gauge were placed on the dominant arm for the measurement of FBF, which was measured for 5-minute intervals every 10 minutes throughout the study. During these 5 minutes, the wrist cuff was inflated to 200−mm Hg pressure to occlude hand flow, and the upper arm cuff was cyclically inflated to 40 mm Hg to occlude venous return. The strain gauge was connected to a single-channel EC-6 Hokanson plethysmograph (Hokanson, Bellevue, WA). It has been previously demonstrated that this method of measurement of FBF does not alter flow, vascular resistance, or sympathoadrenal activity during vehicle infusion [26,27]. Data were continuously recorded on a MacIntosh Quad 4 (Apple Computer, Cupertino, CA) computer via PowerLab (AD Instruments, Grand Junction, CO) and its accompanying software. Forearm blood flow (mL · min^{-1} · 100 mL^{-1} forearm) was calculated from the slope of the signal while the upper arm cuff was inflated and averaged over 5 minutes.

Table 1
Day 1 clamp results in type 1 diabetic and control subjects

Variable	Group	Study	Baseline clamp 1	End clamp 1	Baseline clamp 2	End clamp 2
Glucose (mmol/L)	Type 1 diabetes mellitus	Hypoglycemia	12.3 ± 1.9	2.8 ± 0.1	5.8 ± 0.7	2.7 ± 0.1
		Euglycemia	8.5 ± 1.3	5.8 ± 0.7	6.9 ± 0.4	5.4 ± 0.1
	Control	Hypoglycemia	4.2 ± 0.2	2.7 ± 0.1	5.1 ± 0.4	2.8 ± 0.1
		Euglycemia	4.4 ± 0.1	4.6 ± 0.2	4.6 ± 0.1	4.9 ± 0.3
Epinephrine (pmol/L)	Type 1 diabetes mellitus	Hypoglycemia	240 ± 20	2070 ± 600*		2010 ± 710*
		Euglycemia	260 ± 80	250 ± 60		280 ± 70
	Control	Hypoglycemia	150 ± 40	2980 ± 890*		2890 ± 750*
		Euglycemia	440 ± 280	400 ± 200		460 ± 20
Norepinephrine (nmol/L)	Type 1 diabetes mellitus	Hypoglycemia	1.4 ± 0.2	1.8 ± 0.2*		2.0 ± 0.2*
		Euglycemia	1.4 ± 0.2	1.4 ± 0.2		1.2 ± 0.1
	Control	Hypoglycemia	1.1 ± 0.2	2.0 ± 0.3*		2.1 ± 0.3*
		Euglycemia	1.3 ± 0.1	1.6 ± 0.1		1.4 ± 0.1
Glucagon (ng/L)	Type 1 diabetes mellitus	Hypoglycemia	208 ± 24	170 ± 15		140 ± 15*
		Euglycemia	229 ± 20	192 ± 20		151 ± 16
	Control	Hypoglycemia	244 ± 26	324 ± 40*,**		210 ± 24*
		Euglycemia	263 ± 40	173 ± 18		131 ± 16
Cortisol (nmol/L)	Type 1 diabetes mellitus	Hypoglycemia	630 ± 80	690 ± 80		660 ± 140
		Euglycemia	610 ± 50	390 ± 50		390 ± 50
	Control	Hypoglycemia	470 ± 3	800 ± 80*		720 ± 140*
		Euglycemia	500 ± 140	410 ± 30		300 ± 3

* $P < .05$ vs baseline.

** $P < .05$ vs type 1 diabetes hypoglycemia study.

Table 2
Preinsulin baseline plasma glucose, insulin, and free fatty acid levels in control and type 1 diabetic subjects

	Controls		Type 1 diabetes mellitus		*P* (diabetic vs controls)[a]
	Antecedent euglycemia	Antecedent hypoglycemia	Antecedent euglycemia	Antecedent hypoglycemia	
Arterial glucose (mmol/L)	4.9 ± 0.1	5.1 ± 0.2	8.7 ± 1.8	8.6 ± 0.1	.001
Insulin (pmol/L)	33 ± 4	37 ± 7	175 ± 83	180 ± 48	.038
Free fatty acids (mmol/L)	0.46 ± 0.06	0.41 ± 0.06	0.56 ± 0.18	0.22 ± 0.04	NS
Glycerol (mmol/L)	0.73 ± 0.011	0.63 ± 0.08	0.63 ± 0.14	0.47 ± 0.04	NS
AVDIFF	0.19 ± 0.03	0.11 ± 0.09	0.22 ± 0.07	0.07 ± 0.23	NS
FGU	0.42 ± 0.06	0.28 ± 0.21	0.98 ± 0.46	0.64 ± 1.17	NS

NS indicates not significant.
[a] No differences were present between antecedent euglycemia and antecedent hypoglycemia.

Blood pressure was measured and continuously recorded via a pressure transducer attached to the radial artery catheter. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure by FBF. Heart rate was measured by continuous electrocardiographic monitoring.

2.5. Assays

Plasma glucose concentration was measured immediately using 1 of 2 YSI 2300 Stat Glucose Analyzers (Yellow Springs Instruments, Yellow Springs, OH). Arterial and venous samples were alternately measured on each machine. Plasma norepinephrine, epinephrine, NEFA, glycerol, free insulin, glucagon, and cortisol concentrations were measured in the CORE laboratory of Clinical Research Center of the Ohio State University.

2.6. Statistical analysis

Student *t* tests and paired *t* test were used for comparison of results between groups during the preinsulin and preepinephrine baseline periods. Paired *t* test was used to compare preinsulin and preepinephrine baseline results within each group.

Repeated-measures analysis of variance (ANOVA) was used to compare responses to epinephrine between control

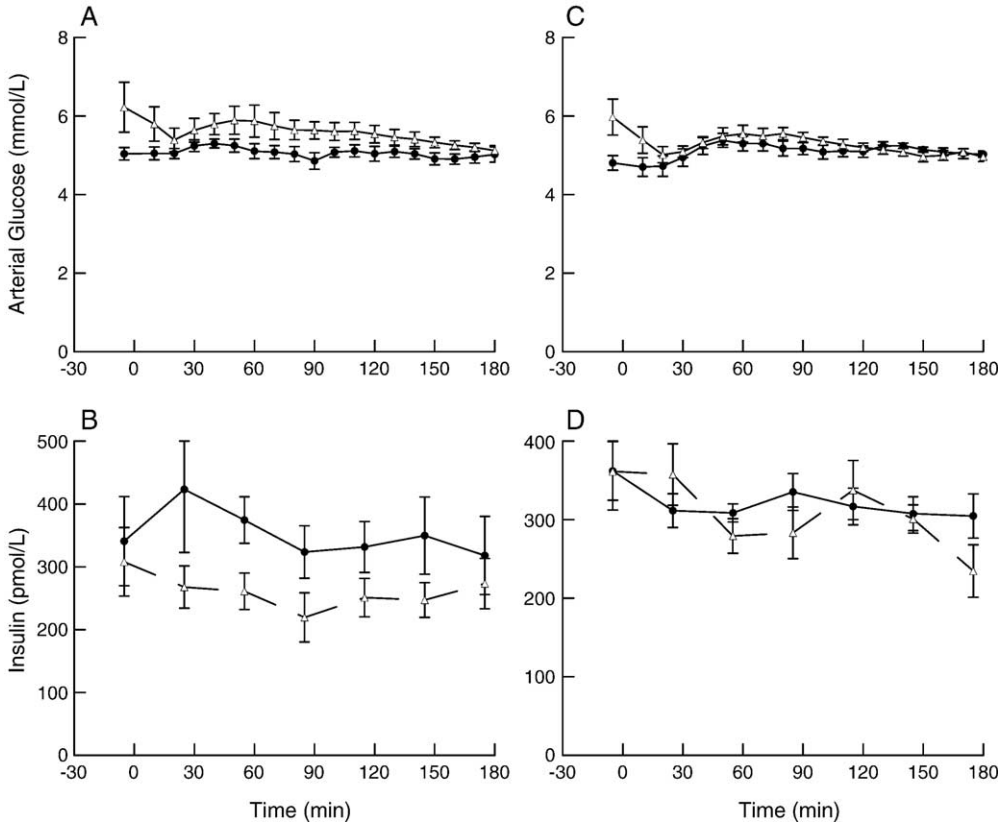

Fig. 2. Arterial plasma glucose (A, C) and venous plasma free insulin levels (B, D) in response to epinephrine infusion (0.015 $\mu g \cdot kg^{-1} \cdot min^{-1}$) beginning at time 0 with antecedent euglycemia (A, B) or hypoglycemia (C, D) on the day before in type 1 diabetic (open triangles, dashed line) and control subjects (solid circles and lines). Insulin infusion (40 $mU \cdot m^{-2} \cdot min^{-1}$) began 60 minutes before starting epinephrine. Glucose levels over time differed between type 1 diabetic and control subjects, but not between studies (time by group interaction, $P < .001$). Insulin levels did differ between groups or studies.

and diabetic subjects. Statistical analysis was performed using repeated ANOVA with 2 grouping factors. The grouping factors were subject group (type 1 diabetes mellitus vs control) and antecedent glycemic level (hypoglycemia vs euglycemia). Student t test and paired t tests were used for post hoc comparisons, as indicated.

Results are reported as mean ± SE. Differences were considered statistically significant if $P < .05$.

3. Results

3.1. Day 1

Table 1 shows the baseline plasma glucose, epinephrine, norepinephrine, glucagon, and cortisol levels before the first insulin clamp and at the end of the first and second insulin clamps on day 1. Glucose levels were significantly higher at baseline in the diabetic subjects ($P < .001$), but were not different between patients with diabetes and controls at the end of either euglycemia or hypoglycemia. Glucagon levels at the end of each hypoglycemic clamp were significantly lower in the diabetic than in control subjects ($P < .001$). Epinephrine, norepinephrine, and cortisol responses did not differ between groups.

3.2. Day 2

3.2.1. Glucose and insulin

Plasma glucose concentrations were significantly higher in type 1 diabetic subjects than in control subjects before starting insulin ($P = .001$; Table 2). Arterial plasma glucose levels were also slightly higher in the type 1 diabetic subjects at the start of epinephrine ($P = .008$). A significant group by time interaction was present in response to starting epinephrine ($P < .001$; Fig. 2). Arterial plasma glucose levels were no different between type 1 diabetic and control subjects over the last 1 hour of the study. There were no differences between antecedent euglycemia and hypoglycemia for either group. Plasma free insulin levels were significantly lower in controls before starting the insulin infusion ($P = .038$). Repeated-

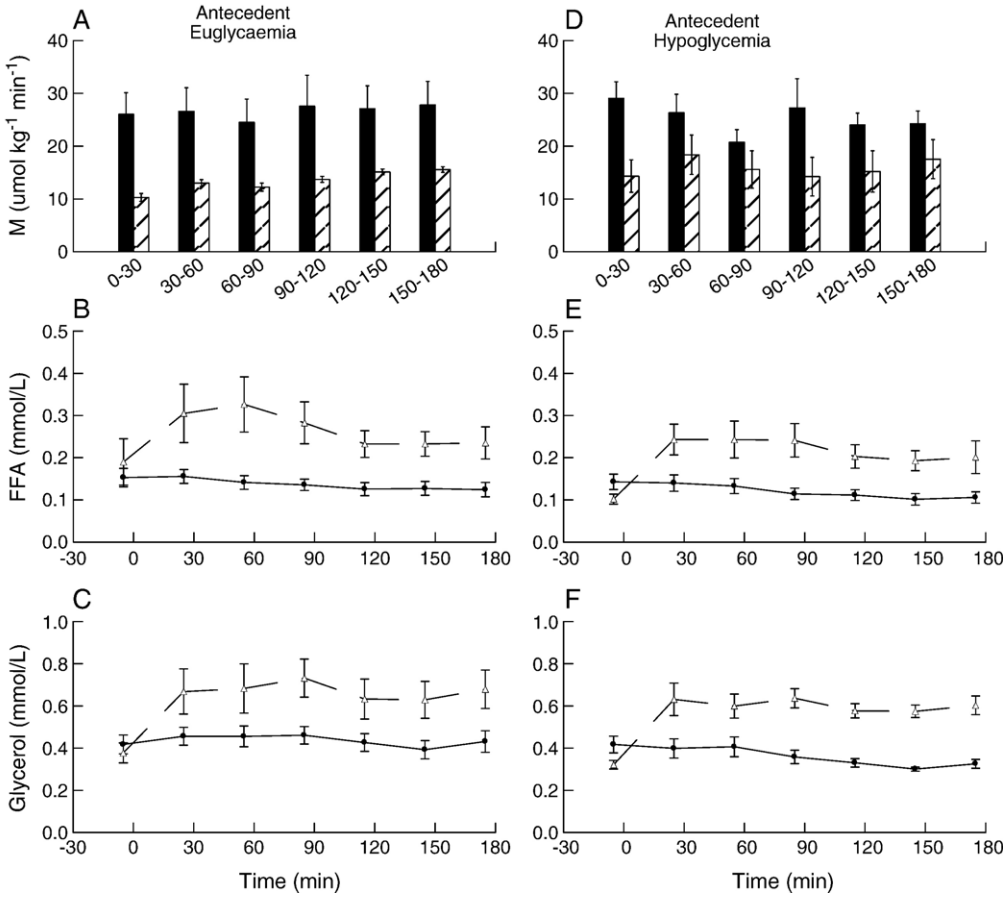

Fig. 3. Total body glucose utilization (A, D), plasma free fatty acid (B, E), and glycerol levels (C, F) in response to epinephrine infusion (0.015 $\mu g \cdot kg^{-1} \cdot min^{-1}$) beginning at time 0 with antecedent euglycemia (A-C) or hypoglycemia (D-F) on the day before in type 1 diabetic (open bars or triangles, dashed line) and control subjects (solid bars or circles and lines). Insulin infusion (40 $mU \cdot m^{-2} \cdot min^{-1}$) began 60 minutes before starting epinephrine. Differences were found between groups for M (group effect, $P = .002$), NEFA (time by group interaction, $P < .001$), and glycerol (time by group interaction, $P < .001$). No differences were found between study sessions.

measures ANOVA revealed no group or study effects during the epinephrine infusion.

3.2.2. Systemic effects

M during epinephrine was significantly ($P = .002$) lower in type 1 diabetic than in control subjects (Fig. 3). Antecedent hypoglycemia did not affect *M* in either group.

Nonesterified fatty acid and glycerol levels did not differ between groups before (Table 2) or after insulin, but were significantly suppressed in both groups (NEFA: control subjects, 0.15 ± 0.02; type 1 diabetic subjects, 0.19 ± 0.06 mmol/L; glycerol: control subjects, 0.42 ± 0.05; type 1 diabetic subjects, 0.38 ± 0.05 mmol/L for antecedent euglycemia) after 60 minutes of insulin before starting epinephrine ($P < .001$) There was no difference in response to insulin between the 2 groups. The increase in free fatty acid levels in response to epinephrine was significantly higher in the type 1 diabetic subjects (time by group interaction, $P < .001$; Fig. 3). The increased lipolytic response to epinephrine was confirmed by the demonstration of an increased glycerol response to epinephrine in type 1 diabetic subjects than in control subjects ($P < .001$; Fig. 3). Both NEFA ($P = .003$) and glycerol levels ($P < .001$) were significantly increased in diabetic subjects 30 minutes after starting epinephrine. No increase was seen in control subjects. Antecedent hypoglycemia did not alter the lipolytic response to epinephrine in either group.

Plasma glucagon levels (Table 3) were suppressed by insulin in both groups (time effect, $P < .0001$) and further fell during epinephrine ($P < .001$), but there were no between-group or study differences in response to insulin or epinephrine. Plasma epinephrine levels were not affected by insulin alone in either group and did differ between type 1 diabetic and control subjects or between antecedent euglycemia and antecedent hypoglycemia at the end of the study. Plasma norepinephrine levels were not affected by insulin alone, but significantly increased during epinephrine infusion ($P < .001$). There were no group or study differences. Plasma cortisol levels did not differ between groups or change with time.

3.2.3. Forearm effects

Glucose AVDIFF did not differ between groups during the preinsulin periods (Table 2), but was slightly lower in the diabetic subjects just before starting epinephrine ($P = .049$; Fig. 4). Repeated-measures ANOVA revealed a significant group by time interaction in response to epinephrine ($P = .009$). Post hoc analysis, however, revealed that the only significant differences between the 2 groups were 25 and 145 minutes after starting epinephrine ($P = .02$). There were no significant differences between antecedent euglycemia and hypoglycemia for either group.

Forearm blood flow was not different between control and type 1 diabetic subjects before insulin and was unaffected by starting insulin. In response to epinephrine, FBF also significantly increased in all studies (time effect, $P < .001$), and the increase was significantly greater in type 1 diabetic subjects after antecedent hypoglycemia (time by group by study interaction, $P = .012$). In all subjects, FBF was increased 5 minutes after starting epinephrine ($P < .001$; Fig. 4) and remained increased until the end of the study. Forearm blood flow levels were significantly higher in type 1 patients with diabetes after antecedent hypoglycemia beginning 115 minutes after starting epinephrine and continuing through the end of the study.

Forearm glucose uptake did not differ between groups or studies before insulin (Table 2). With antecedent

Table 3
Day 2 counterregulatory hormones before insulin infusion, after 60 minutes of insulin infusion (40 mU · m^{-2} · min^{-1}) alone, and after 180 minutes of epinephrine infusion (0.015 μg · kg^{-1} · min^{-1}) with euglycemic hyperinsulinemic clamp

Variable	Group	Day 1 study	Preinsulin (−60 min)	Insulin alone (0 min)	Insulin plus epinephrine (180 min)	P[a]	
						−60 vs 0 min	0 vs 180 min
Glucagon (ng/L)	Type 1 diabetes mellitus	Hypoglycemia	178 ± 16	156 ± 16	138 ± 15	<.001	<.001
		Euglycemia	192 ± 33	182 ± 23	283 ± 39		
	Control	Hypoglycemia	246 ± 42	202 ± 34	168 ± 28		
		Euglycemia	229 ± 24	186 ± 15	149 ± 17		
Cortisol (nmol/L)	Type 1 diabetes mellitus	Hypoglycemia	582 ± 93	472 ± 88	459 ± 80	NS	NS
		Euglycemia	509 ± 53	521 ± 32	389 ± 47		
	Control	Hypoglycemia	345 ± 88	460 ± 71	336 ± 104		
		Euglycemia	355 ± 76	469 ± 44	405 ± 84		
Epinephrine (pmol/L)	Type 1 diabetes mellitus	Hypoglycemia	226 ± 36	207 ± 22	1780 ± 125	NS	<.001
		Euglycemia	170 ± 38	266 ± 82	2010 ± 161		
	Control	Hypoglycemia	180 ± 38	168 ± 15	1580 ± 118		
		Euglycemia	124 ± 31	148 ± 19	1960 ± 213		
Norepinephrine (nmol/L)	Type 1 diabetes mellitus	Hypoglycemia	1.1 ± 0.2	1.1 ± 0.1	1.7 ± 0.2	NS	<.001
		Euglycemia	0.9 ± 0.1	1.2 ± 0.2	1.5 ± 0.2		
	Control	Hypoglycemia	0.8 ± 0.2	1.0 ± 0.1	1.5 ± 0.2		
		Euglycemia	1.3 ± 0.1	1.3 ± 0.2	2.0 ± 0.4		

[a] There were no significant group or study effects, or time by group or study interactions.

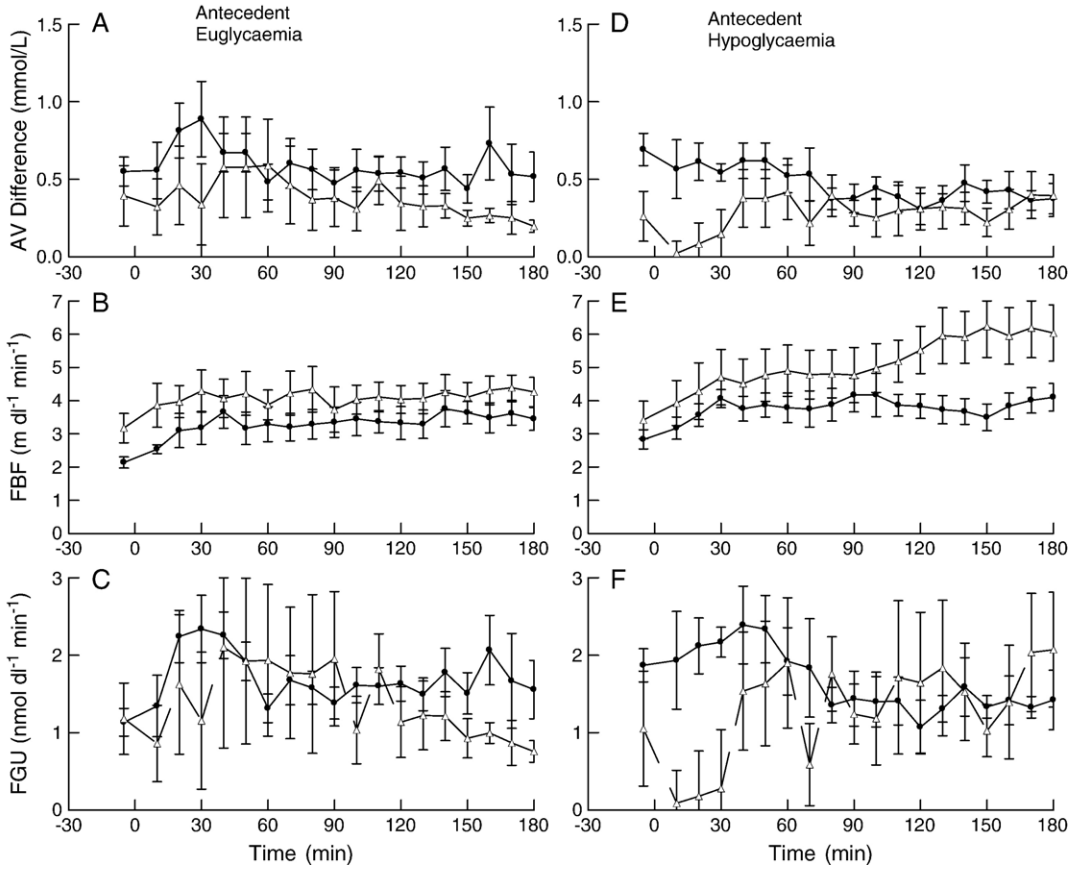

Fig. 4. Arterial venous glucose difference (A, D), FBF (B, E), and FGU (D, F) in response to epinephrine infusion (0.015 $\mu g \cdot kg^{-1} \cdot min^{-1}$) beginning at time 0 with antecedent euglycemia (A-C) or hypoglycemia (D-F) on the day before in type 1 diabetic (open triangles, dashed line) and control subjects (solid circles and lines). Insulin infusion (40 $mU \cdot m^{-2} \cdot min^{-1}$) began 60 minutes before starting epinephrine. Differences were found between groups for AVDIFF (time by group interaction, $P = .009$), FBF (time effect, $P < .001$), and FGU (time by group interaction, $P = .019$). The FBF increase in type 1 diabetic subjects after antecedent hypoglycemia was significantly greater than other responses (time by group by study interaction, $P = .012$).

euglycemia, FGU increased with insulin infusion alone in control subjects (1.13 ± 0.17 nmol $\cdot$ $dL^{-1} \cdot min^{-1}$, $P < .001$), but did not increase in type 1 diabetic subjects (0.79 ± 0.37 nmol $\cdot$ $dL^{-1} \cdot min^{-1}$). Changes were similar after antecedent hypoglycemia. In response to epinephrine, the time by group interaction was significant ($P = .019$; Fig. 4). Post hoc comparisons, however, revealed the only significant difference to be 30 minutes after starting epinephrine ($P = .021$).

3.2.4. Relationship between systemic and forearm effects

Stepwise multiple linear regression with NEFA, FGU, insulin, cortisol, glucagon, and epinephrine levels as potential independent variables and M as the dependent variable revealed that at the end of the study on day 2 the equation $M = 4.4 + 3.2$ (FGU) $- 10.6$ (NEFA) significantly predicted total body glucose utilization ($R^2 = 0.37$, $P < .004$). Both relationships were significant ($P < .03$). Similar results were found when type 1 diabetic and control subjects were analyzed separately, although in the type 1 diabetic subjects FGU was no longer included.

3.2.5. Cardiovascular responses

Heart rate significantly varied with time ($P = .001$; Table 4) with significant increases present in all subjects 30 minutes after starting epinephrine ($P = .021$). Heart rate was higher in type 1 diabetic subjects ($P = .006$), but the response to epinephrine did not differ between groups. Antecedent hypoglycemia had no effect. No significant group or study differences were seen for mean arterial pressure or FVR. Forearm vascular resistance was significantly reduced 30 minutes after starting epinephrine ($P = .014$).

4. Discussion

The current study indicates that patients with type 1 diabetes mellitus and variable glucose control have increased β-adrenergic lipolytic response. This increased lipolytic response may compensate for decreased epinephrine secretion and was demonstrated by increased NEFA and glycerol levels throughout a 3-hour epinephrine infusion with euglycemic clamp. Importantly, epinephrine concentrations during the epinephrine infusion were similar

to those during moderate hypoglycemia in the type 1 diabetic subjects, indicating that the results are physiologically relevant. The moderate hyperinsulinemia present during the insulin clamp on day 2 is likely similar to that present in diabetic patients during routine hypoglycemic reactions (Table 4).

Whether differences in lipolytic response to β-adrenergic stimulation between type 1 diabetic and control subjects exist is a controversial area. Some studies found no differences in response to direct adrenergic stimulation [24,28] or response to hypoglycemic adrenergic counterregulation, whereas others have found significant increases in lipolytic [29] or ketogenic [30] responses to epinephrine infusion or hypoglycemia-induced epinephrine secretion in type 1 diabetes mellitus [31,32]. Differences in metabolic control, method of β-adrenergic stimulation, and metabolic conditions likely account for the differences. In one study, higher insulin infusion rates and higher plasma glucose levels in the diabetic subjects may have led to greater lipolytic suppression and potentially masked differences [24,33]. In the other study finding no difference, the use of local infusion of the strict β_2-adrenergic agonist terbutaline at high doses [28] may have hidden differences between type 1 diabetic and control subjects at submaximal stimulation [34].

In contrast to the results from the current study that indicate an increased lipolytic response to epinephrine, decreased β-adrenergic sensitivity is suggested in 2 studies that found right-shifted dose-response curves for heart rate increase to isoproterenol in type 1 diabetic subjects [9,10]. The contrasting results, again, may be due to differences in subjects studied and method of adrenergic stimulation. These studies specifically examined subjects with hypoglycemic unawareness and used multiple doses of isoproterenol, a strictly β-adrenergic stimulant. In the current study, subjects with recent severe hypoglycemia were excluded and only one dose of epinephrine was used. Dose-response curves were not generated. In addition, cardiac and metabolic responsiveness may differ within a given individual. This is clearly demonstrated by the lack of differences between control and type 1 diabetic subjects in heart rate response to epinephrine in the current study.

The negative relationship found between NEFA levels and M suggests that increased rate of lipolysis during epinephrine in the diabetic subjects is likely responsible for the decreased glucose utilization. This appears to be because of lower muscle glucose utilization as revealed by the lower FGU in the type 1 diabetic subjects as a whole, although differences between individual time points were not routinely present. Interestingly, however, free fatty acid levels remained negatively correlated to glucose utilization even when FGU was included in the equation. Previous data in control subjects found a negative correlation between NEFA levels and muscular glucose extraction [23]. This relationship was not present in the current study. This may be due to an inadequate sample size. This is particularly likely to be true regarding muscular glucose uptake because FBF comparisons between subjects are dependent on cuff and strain gauge placement. In support of a role for lipolysis in decreasing glucose utilization during hypoglycemia, the glycerol response to hypoglycemia is negatively correlated to whole-body glucose disposal [33]. It is thus likely that the increased lipolytic fuel available decreases muscular glucose utilization during hypoglycemia.

Increases in α-adrenergic stimulation suppress lipolysis [35], whereas increases in β-adrenergic activity stimulate lipolysis. The increased NEFA and glycerol levels in type 1 diabetic subjects in the current study could be due to either a diminished α-adrenergic response or an increased β-adrenergic response. It is also possible that the increased response may not be due directly to epinephrine's action on lipolysis, but to an alteration of lipolytic suppression by insulin. Hyperinsulinemic suppression of the ketogenic response to epinephrine is reduced in type 1 diabetic subjects [30]. The fact that insulin similarly suppressed free fatty acid and glycerol levels in both groups before

Table 4
Day 2 cardiovascular measures before insulin infusion, after 60 minutes of insulin infusion (40 mU · m^{-2} · min^{-1}) alone, and after 180 minutes of epinephrine infusion (0.015 μg · kg^{-1} · min^{-1}) with euglycemic insulinemic clamp

Variable	Group	Day 1 study	Preinsulin (−60 min)	Insulin alone (0 min)	Insulin plus epinephrine (180 min)	P[a]		
						−60 vs 0 min	0 vs 180 min	Diabetic vs control
Heart rate (beats per min)	Type 1 diabetes mellitus	Hypoglycemia	70 ± 3	74 ± 3	77 ± 4	NS	.004	.001
		Euglycemia	72 ± 3	73 ± 3	80 ± 3			
	Control	Hypoglycemia	61 ± 4	67 ± 7	71 ± 5			
		Euglycemia	67 ± 7	58 ± 4	64 ± 4			
Mean arterial pressure (mm Hg)	Type 1 diabetes mellitus	Hypoglycemia	86 ± 2	85 ± 3	90 ± 5	NS	NS	NS
		Euglycemia	75 ± 7	77 ± 7	81 ± 7			
	Control	Hypoglycemia	86 ± 2	90 ± 2	91 ± 2			
		Euglycemia	89 ± 2	89 ± 2	89 ± 3			
FVR (mm Hg · mL^{-1} · dL^{-1} · min^{-1})	Type 1 diabetes mellitus	Hypoglycemia	27 ± 4	25 ± 3	18 ± 4	NS	<.001	NS
		Euglycemia	26 ± 5	29 ± 6	20 ± 2			
	Control	Hypoglycemia	34 ± 4	34 ± 4	23 ± 3			
		Euglycemia	40 ± 2	44 ± 3	27 ± 3			

[a] There were no significant study, time by group, or time by study effects.

epinephrine infusion was started makes it unlikely that direct differences in insulin sensitivity are responsible for the differences in free fatty acid and glycerol levels during epinephrine. Similarly, this fact makes it unlikely that the differences are due to the preinsulin differences in plasma glucose levels between the 2 groups.

It is also possible that lower clearance rates and not increased production are responsible for the higher glycerol and NEFA levels in the type 1 diabetic subjects. This is unlikely for 3 reasons. First, there is no evidence that epinephrine affects clearance rates. Second, difference in clearance rates should have been manifested by increased baseline levels. Third, the fact that both NEFA and glycerol were increased strongly supports an increased lipolytic response.

A mechanism for the increased lipolytic response to epinephrine in the type 1 diabetic subjects is not readily apparent. One possibility is subclinical autonomic neuropathy in type 1 diabetes mellitus because muscle sympathetic nerve activity levels are decreased before overt autonomic neuropathy becomes apparent [36]. The loss of sympathetic fibers could lead to decreased epinephrine reuptake.

The current study clearly indicates that antecedent hypoglycemia has no effect on the lipolytic response to epinephrine in either group. The only effect of two 2-hour episodes of hypoglycemia on day 1 was to increase epinephrine's vasodilatory effect in the type 1 diabetic subjects. Antecedent hypoglycemia had no effect on total body or forearm glucose utilization, or free fatty acid or glycerol levels in either group. This is in contrast to studies [11,12] that report that antecedent hypoglycemia increased cardiac β-adrenergic sensitivity in control subjects and decreased sensitivity in type 1 diabetic subjects. The difference, again, could be due to different adrenergic agents used or to exclusion of subjects with recent severe hypoglycemia in the current study. It is also possible that differences in the way antecedent hypoglycemia was induced may play a role because these studies used a single nocturnal episode compared with more prolonged hypoglycemia in the current study. The more prolonged hypoglycemia induced twice on day 1 was justified by the fact that if increased β-adrenergic sensitivity is to compensate for decreased epinephrine response, it is desirable to induce antecedent hypoglycemia in a way that has been documented to reduce the epinephrine response. The protocol in the current study has been shown to do so [22].

The current study has several limitations. First, higher plasma free insulin levels could explain the absence of a lipolytic response in the control subjects after antecedent euglycemia, although the differences were not statistically significant. The reason for the higher insulin levels is unclear because the insulin infusion rates were identical. After antecedent hypoglycemia, however, free insulin levels were identical in the 2 groups, yet the NEFA and glycerol levels significantly increased during epinephrine in type 1 diabetic, but not control subjects, and were again higher in the former group. There were no differences in other counterregulatory hormones. The slightly higher glucose levels in the type 1 diabetic subjects are also unlikely to explain the increased lipolysis because hyperglycemia in combination with hyperinsulinism suppresses lipolysis in type 1 diabetes mellitus. The fact that both females and males were studied and that lipolytic responses to epinephrine differ between sexes [32] may also confound the current results. Exclusion of the female subjects in the current study does not alter the results.

In conclusion, subjects with type 1 diabetes mellitus have increased lipolytic responses to epinephrine that are associated with decreased total body glucose utilization. Antecedent hypoglycemia increased the vasodilatory response to epinephrine in type 1 diabetic subjects, but did not alter the metabolic responses to physiological doses of epinephrine in control or diabetic subjects. This increased response may partially compensate for the decreased epinephrine responses to hypoglycemia seen in patients with type 1 diabetes mellitus.

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