

Hypoglycemic Counterregulatory Responses Differ Between Men and Women With Type 1 Diabetes

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The aim of this study was to determine whether sex-related differences occur in counterregulatory responses to hypoglycemia in adult type 1 diabetic patients. Experiments were carried out on 16 (8 men/8 women) type 1 diabetic patients and compared with 16 (8 men/8 women) age- and weight-matched normal individuals. Men and women with type 1 diabetes were matched for age (26 ± 2 vs. 25 ± 1 years), duration of diabetes (9 ± 1 vs. 8 ± 1 years), glycemic control (HbA_{1c} 7.7 ± 0.3 vs. $7.8 \pm 0.2\%$), and weight (BMI 22.8 ± 1 vs. 22.1 ± 1 kg/m²), respectively. After normalizing plasma glucose overnight, patients underwent a 2-h hyperinsulinemic-hypoglycemic clamp study. Plasma glucose (3.0 ± 0.1 mmol/l) and insulin (510 ± 48 pmol/l) levels were equated in all groups. Plasma epinephrine, norepinephrine, growth hormone (GH), muscle sympathetic nerve activity (MSNA), and endogenous glucose production (EGP) responses were significantly lower ($P < 0.01$) in type 1 diabetic women compared with men. Autonomic symptom scores, lipid oxidation, nonesterified fatty acids (NEFAs), and glycerol responses were equivalent between men and women with type 1 diabetes despite significantly reduced sympathoadrenal and MSNA responses in women. Autonomic nervous system (ANS) and EGP responses were equivalent in type 1 diabetic and normal individuals. However, lipid oxidation (assessed by indirect calorimetry), glycerol, and NEFA responses were increased ($P < 0.01$) in type 1 diabetic patients compared with normal control subjects. We conclude that counterregulatory responses to fixed hypoglycemia differ markedly in men and women with type 1 diabetes: 1) sympathetic nervous system, GH, and EGP responses are significantly reduced in type 1 diabetic women, 2) autonomic symptom awareness and lipolytic responses appear to be relatively increased in type 1 diabetic women compared with men, and 3) during conditions of similar hyperinsulinemic hypoglycemia and ANS drive, lipid oxidation and lipolytic responses can be increased in type 1 diabetic patients compared with normal individuals. *Diabetes* 49:65–72, 2000

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ANS, autonomic nervous system; AUC, area under the curve; CV, coefficient of variation; dBp, diastolic blood pressure; EGP, endogenous glucose production; EPI, epinephrine; GH, growth hormone; HPLC, high-pressure liquid chromatography; MSNA, muscle sympathetic nerve activity; NE, norepinephrine; NEFA, nonesterified fatty acid; R_a , rate of glucose appearance; R_d , rate of glucose disposal; RIA, radioimmunoassay; sBP, systolic blood pressure; SNS, sympathetic nervous system.

Recent studies have demonstrated that a wide spectrum of neuroendocrine counterregulatory responses to hypoglycemia are reduced in healthy young women (1–4). Included in this array of altered responses are the key counterregulatory hormones glucagon and epinephrine (EPI). These findings tend to explain why plasma glucose levels fall to a lower level during fasting in women (5). If a similar spectrum of reduced neuroendocrine counterregulatory responses exist in women with type 1 diabetes, then an increased incidence of hypoglycemia might be expected in these individuals. However, data from the Diabetes Control and Complications Trial demonstrate that the frequency of hypoglycemia is sex neutral in type 1 diabetes (6). This finding appears somewhat incongruous with data on normal women and poses the question whether counterregulatory responses to hypoglycemia are similar in healthy and type 1 diabetic women. To date, virtually no data are available examining the effects of sex on hypoglycemic counterregulation in type 1 diabetic adults.

Arslanian et al. (7) investigated the effects of sex in a group of adolescents with type 1 diabetes. Interestingly, these authors reported that neuroendocrine counterregulatory responses were similar or even increased (growth hormone [GH]) in adolescent females with type 1 diabetes compared with age-matched type 1 diabetic males. In the study of Arslanian et al., glucose levels were not controlled but allowed to fall freely after cessation of a hyperinsulinemic-euglycemic clamp. Because of differences in insulin sensitivity, glucose levels were significantly lower in males, and thus a comparison of counterregulatory responses at equivalent hypoglycemia between sexes appears to be lacking in type 1 diabetic patients. Therefore, the aim of this study is to determine whether neuroendocrine, metabolic, and autonomic nervous system (ANS) responses to fixed equivalent hypoglycemia differ in men and women with type 1 diabetes.

RESEARCH DESIGN AND METHODS

We studied 16 type 1 diabetic patients (8 M/8 F) matched for age, BMI, HbA_{1c} , and duration of diabetes (Table 1) and 16 (8 M/8 F) age- and weight-matched healthy control subjects. Normal control subjects were 19–37 years of age (26 ± 2 years) and had a mean BMI of 22.9 ± 0.7 kg/m². None of the patients gave a history of hypoglycemic unawareness and all received insulin as their only regular medication. No patient had any clinical evidence of autonomic neuropathy or any other tissue-specific complication of diabetes. All patients and control subjects tested normal for blood count, plasma electrolytes, and liver and renal function. All gave written informed consent. The study was approved by the local ethical committee. Patients were asked to consume their usual weight-maintaining diet for 3 days before a study. We instructed each patient to be particularly careful to avoid any hypoglycemia in the period before a study. All patients performed intensive self-monitoring of blood glucose (before each meal, at bedtime, and on two occasions

TABLE 1
Clinical characteristics of type 1 diabetic patients

	Men	Women
Age (years)	26 ± 2	25 ± 1
BMI (kg/m ²)	22.8 ± 1	22 ± 1
HbA _{1c} (NR 4–6.5%)	7.7 ± 0.3	7.8 ± 0.2
Duration of diabetes (years)	9 ± 1	8 ± 1
Insulin replacement regimen		
Multiple daily injections	5	4
Insulin infusion pump	1	2
Twice-daily short- and intermediate-acting insulin	2	2

Data are means ± SE or n. NR, normal range.

at 3:00 A.M.) for 2 weeks before a study. An experiment was not conducted unless all readings were 4.5 mmol/l or more. On the day preceding an experiment, intermediate-acting insulin was discontinued and replaced by injections of regular insulin before breakfast and lunch. Each patient was admitted to the Vanderbilt University Clinical Research Center at 5:00 P.M. on the evening before an experiment. At this time, the patients received their usual evening meal, and a continuous low-dose intravenous infusion of insulin was started so that the patients' plasma glucose could be normalized. This infusion of insulin was adjusted so that plasma glucose levels remained between 5.0 and 6.7 mmol/l overnight. On no occasion was the plasma glucose level allowed to drop below 5 mmol/l during the night. All patients and healthy control subjects were studied after an overnight 10-h fast.

Experimental design

Glucose clamp studies. On the morning of each study, after an overnight fast, two intravenous cannulae were inserted under 1% lidocaine local anesthesia. One cannula was placed in a retrograde fashion into a vein on the back of the hand. The hand was placed in a heated box (60°C), so that arterialized blood could be obtained. The other cannula was placed in the contralateral arm so that 20% glucose could be infused via a variable rate volumetric infusion pump (IMED, San Diego, CA). Purified tritiated glucose and insulin were infused into this cannula via precalibrated infusion pumps (Harvard Apparatus, South Natick, MA).

Each experiment consisted of a tracer equilibration period (0–90 min), a control period (90–120 min), and an experimental period (120–240 min). A priming dose of [³-H]glucose (20 µCi) was given in a logarithmically decreasing manner over 10 min starting at time 0 min. A constant infusion of [³-H]glucose (0.2 µCi/min) was also started at 0 min. The overnight infusion of insulin was continued and adjusted during the initial isotope equilibration and control periods so that plasma glucose was maintained in the normal range. At 120 min, a primed continuous infusion of insulin (9 pmol · kg⁻¹ · min⁻¹) was administered in a randomized single-blind fashion for a further 120 min. The priming dose was given over the first 10 min in a logarithmic decreasing manner to acutely raise the hormone concentration to the desired level (8). The rate of fall of glucose (0.06 mmol/min) and the hypoglycemic nadir (3.0 ± 0.1 mmol · l⁻¹ · dl⁻¹) were equated in both groups by a modification of the glucose clamp technique (9). Plasma glucose levels were measured every 5 min, and a variable infusion of 20% dextrose (verified in each study) was adjusted so that the desired plasma glucose level was achieved. KCl (20 mmol/l) was added to the glucose infusate in each experiment.

Rates of glucose appearance (R_a), endogenous glucose production (EGP), and glucose utilization were calculated according to the methods of Wall et al. (10). EGP was calculated by determining the total R_a (this comprises both EGP and any exogenous glucose infused to maintain the desired hypoglycemia) and subtracting from it the amount of exogenous glucose infused. It is now recognized that this approach is not fully quantitative, since underestimates of total R_a and rate of glucose disposal (R_d) can be obtained. The use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminate most, if not all, of the problems.

Direct measurement of muscle sympathetic nerve activity. Microneurographic activity was recorded from the peroneal nerve at the level of the fibular head (11). The approximate location of this nerve was determined by transdermal electrical stimulation (10–60 V, 0.01 ms duration). This stimulation produced painless muscle contraction of the foot. After this, a reference tungsten electrode with a shaft diameter of 200 µm was placed subcutaneously. A similar electrode, with an uninsulated tip (1–5 µm) was inserted into the nerve and used for recording of muscle sympathetic nerve activity (MSNA). Placement of the recording electrode was guided by electrical stimulation (1–4 V, 0.01 ms duration).

Recording signals were fed to a preamplifier (1,000-fold amplification) and filtered using a band width between 700 and 2,000 Hz. The filtered signal was rectified, amplified a further 100-fold, and integrated in a resistance-capacitance network using a time constant of 0.1 s (Nerve Traffic Analysis System 662C-3; University of Iowa Bioengineering, Iowa City, IA). The final signal was monitored using a storage oscilloscope (Tefronics S111A, Beaveston, OR) and recorded after fourfold amplification on a Gould TA-2000 recorder (Gould, Oxnard, CA). A recording of MSNA was considered adequate when 1) electrical stimulation produced muscle twitches but not paresthesia, 2) stretching of the tendons in the foot evoked proprioceptive afferent signals, whereas cutaneous stimulation by slight stroking of the skin did not, 3) nerve activity increased during phase II of the Valsalva maneuver (hypotensive phase) and was suppressed during phase IV (blood pressure overshoot), and 4) nerve activity increased in response to held expiration. MSNA is expressed as bursts/min. Measurements of MSNA were made from the original tracing using a digitizer tablet (HIPAD; Houston Instruments, Austin, TX) coupled to Sigma Scan Software (Jandel Scientific, Coite Modena, CA) in a microcomputer. The effect of hypoglycemia on MSNA was expressed as the change (Δ) from the preceding control period.

Two types of sympathetic fibers (skin and muscle) can be identified from recordings of peripheral nerves. MSNA was recorded in the present studies, since this has been demonstrated to reflect increased sympathetic activity during insulin-induced hypoglycemia (12), 2-deoxyglucose-induced neuroglycopenia (13), and hyperinsulinemic euglycemia in normal humans (14).

Indirect calorimetry. Air flow and O₂ and CO₂ concentrations in expired and inspired air were measured by a computerized open-circuit system (Delta Trak; SensorMedics, Yorba Linda, CA). Urea nitrogen was measured by the Kjeldahl procedure (15). Rates of carbohydrate and fat oxidation were calculated from O₂ consumption and CO₂ production (corrected for protein oxidation) with the equations described by Frayn (16).

Analytical methods. The collection and processing of blood samples have been described elsewhere (17). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured according to a modification of the method of Morgan and Lazarow (18) with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (19) with an interassay CV of 9%. Catecholamines were determined by high-pressure liquid chromatography (HPLC) (20) with an interassay CV of 12% for EPI and 8% for norepinephrine (NE). We made two modifications to the procedure for catecholamine determination: 1) we used a five-point rather than a one-point standard calibration curve, and 2) we spiked the initial and final samples of plasma with known amounts of EPI and NE so accurate identification of the relevant respective catecholamine peaks could be made. Cortisol was assayed by using the clinical assays Gamma Coat radioimmunoassays (RIAs) kit with an interassay CV of 6%. GH was determined by RIA (21) with a CV of 8.6%. Pancreatic polypeptide was measured by RIA using the method of Hagopian et al. (22) with an interassay CV of 8%. Lactate, glycerol, alanine, and β-hydroxybutyrate were measured in deproteinized whole blood using the method of Lloyd et al. (23). Nonesterified fatty acids (NEFAs) were measured using the Wako kit (Wako, Richmond, VA) adopted for use on a centrifugal analyzer (24).

Blood samples for glucose flux were taken every 10 min throughout the control period and every 15 min during the experimental period. Blood for hormones and intermediary metabolites were drawn twice during the control period and every 15 min during the experimental period. Cardiovascular parameters (pulse, systolic blood pressure [sBP], diastolic blood pressure [dBP], and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each 240-min study. MSNA was measured continuously throughout each 240-min study. Hypoglycemic symptoms were quantified using a previously validated semiquantitative questionnaire (25). Each study subject was asked to rate his/her experience of the symptoms twice during the control period and every 15 min during the experimental period. Symptoms measured included tiredness, confusion, hunger, dizziness, difficulty thinking, blurred vision, sweating, tremor, agitation, hot/thirsty, and pounding heart. The ratings of the first six symptoms were summed to get a neuroglycopenic score, while the ratings from the last five symptoms provide an autonomic symptom score. Indirect calorimetry measurements were performed during the control period and during the final 30 min of each hypoglycemic clamp experiment.

Materials. HPLC-purified [³-H]glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (11.5 mCi/mmol/l). Human regular insulin was purchased from Eli Lilly (Indianapolis, IN). The insulin infusion solution was prepared with normal saline and contained 3% (vol/vol) of the subject's own plasma.

Statistical analysis. Data are expressed as means ± SE unless otherwise stated. Data were analyzed using standard parametric two-way analysis of variance with a repeated measures design. This was coupled with a paired or unpaired Student's *t* test to delineate at which time statistical significance was reached. A *P* value of <0.05 indicated significant difference.

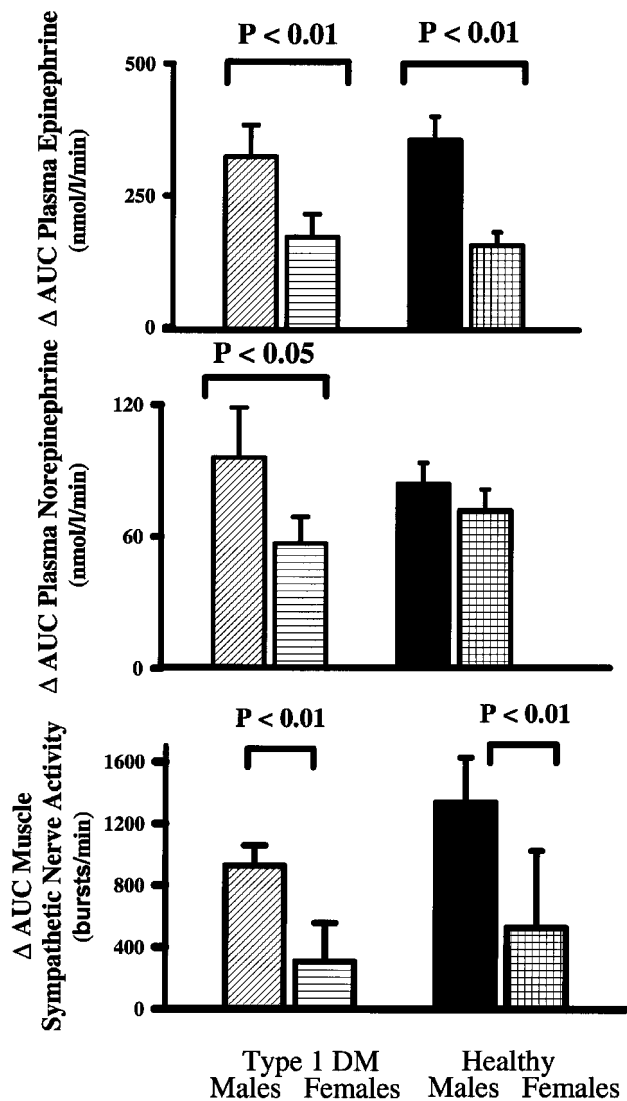


FIG. 1. Effects of peripherally infused insulin ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and hypoglycemia ($3.0 \pm 0.1 \text{ mmol/l}$) on incremental AUC arterialized plasma EPI, NE, and MSNA responses in overnight-fasted type 1 diabetic (type 1 DM) and healthy humans. AUC values for plasma EPI, NE, and MSNA are significantly increased ($P < 0.01$) in type 1 diabetic men compared with women. Incremental AUC values for plasma EPI and MSNA are also increased ($P < 0.01$) in healthy men compared with women.

RESULTS

Insulin and glucose levels. Insulin and glucose levels were similar in all groups at the start of hypoglycemic experiments. Plasma insulin values reached similar steady-state levels ($480 \pm 56 \text{ pmol/l}$) by 30 min in all groups. Plasma glucose fell at similar rates ($0.06 \pm 0.01 \text{ mmol/l}$) in all studies and reached a similar steady-state hypoglycemic plateau of $3.0 \pm 0.1 \text{ mmol/l}$. Stability of the plasma glucose level during the final 45 min of each protocol was demonstrated by a CV of 2.0%.

Neuroendocrine hormones. In response to hypoglycemia, plasma levels of EPI, NE, cortisol, GH, and pancreatic polypeptide increased from baseline ($P < 0.01$). Glucagon values did not increase in response to hypoglycemia in type 1 diabetic patients (Fig. 2). Despite similar hypoglycemia, there were significant sex-related differences in incremental area

TABLE 2
Effects of hyperinsulinemic hypoglycemia on counterregulatory hormones in overnight-fasted type 1 diabetic and normal humans

	Basal	Final 30 min of clamped hypoglycemia
Plasma EPI (nmol/l)		
Type 1 diabetic men	0.27 ± 0.04	$5.0 \pm 0.7^*$
Type 1 diabetic women	0.23 ± 0.05	2.6 ± 0.5
Control men	0.19 ± 0.04	$5.2 \pm 0.5^\ddagger$
Control women	0.23 ± 0.04	2.7 ± 0.4
Plasma NE (nmol/l)		
Type 1 diabetic men	1.1 ± 0.2	$2.3 \pm 0.4^*$
Type 1 diabetic women	1.0 ± 0.1	1.7 ± 0.2
Control men	1.1 ± 0.1	2.1 ± 0.2
Control women	1.2 ± 0.3	1.9 ± 0.2
Plasma glucagon (ngl/l)		
Type 1 diabetic men	47 ± 5	63 ± 18
Type 1 diabetic women	52 ± 8	58 ± 12
Control men	57 ± 9	$298 \pm 28^\ddagger$
Control women	60 ± 12	$148 \pm 39^\ddagger$
Plasma cortisol (nmol/l)		
Type 1 diabetic men	304 ± 55	552 ± 55
Type 1 diabetic women	331 ± 55	607 ± 110
Control men	221 ± 55	$690 \pm 165^\ddagger$
Control women	276 ± 55	$745 \pm 165^\ddagger$
Plasma GH ($\mu\text{g/l}$)		
Type 1 diabetic men	3 ± 1	$25 \pm 5^*$
Type 1 diabetic women	5 ± 2	15 ± 3
Control men	2 ± 1	$46 \pm 6^\ddagger$
Control women	4 ± 1	$31 \pm 5^\ddagger$
Plasma pancreatic polypeptide (pmol/l)		
Type 1 diabetic men	18 ± 3	224 ± 52
Type 1 diabetic women	22 ± 5	235 ± 53
Control men	19 ± 5	$286 \pm 36^\ddagger$
Control women	32 ± 16	165 ± 39

Data are means \pm SE. * $P < 0.05$; type 1 diabetic men values are significantly increased compared with type 1 diabetic women values. $^\ddagger P < 0.05$; control men values are significantly increased compared with control women values. $^\ddagger P < 0.05$; control men and women values are significantly increased compared with type 1 diabetic men and women values.

under the curve (AUC) responses for EPI and NE ($P < 0.01$). EPI responses (Fig. 1, Table 2) were significantly reduced in type 1 diabetic women compared with men (171 ± 44 vs. $324 \pm 55 \text{ nmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). Incremental AUC NE responses were also reduced in type 1 diabetic women compared with men (57 ± 12 vs. $101 \pm 20 \text{ nmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). EPI and NE responses in healthy subjects were also significantly reduced in women relative to men. However, EPI and NE responses were similar between type 1 diabetic and healthy control subjects (Fig. 1, Table 2).

There were no sex-related differences in incremental AUC values for plasma cortisol (Fig. 2, Table 2) in type 1 diabetic or healthy control subjects. Incremental AUC values for cortisol were, however, increased ($\sim 50\%$, $P < 0.01$) in healthy control subjects compared with type 1 diabetic patients. Incremental AUC GH responses (Fig. 2, Table 2) were increased in type 1 diabetic men compared with women ($1,510 \pm 289$ vs.

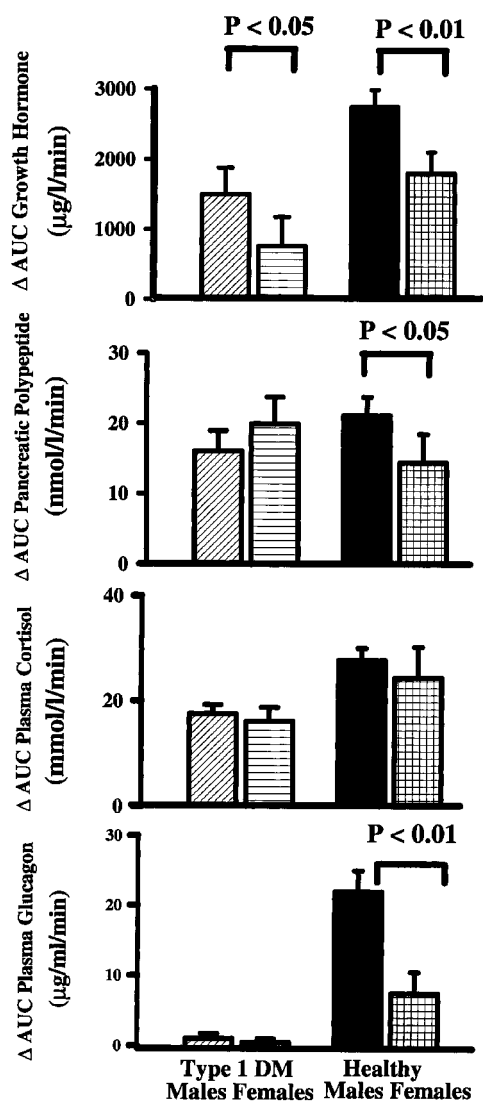


FIG. 2. Effects of peripherally infused insulin ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and hypoglycemia ($3.0 \pm 0.1 \text{ mmol/l}$) on incremental AUC arterialized plasma cortisol, GH, pancreatic polypeptide, and glucagon levels in overnight-fasted type 1 diabetic (type 1 DM) and healthy humans. AUC values for GH are significantly increased ($P < 0.01$) in type 1 diabetic and healthy men compared with women. Pancreatic polypeptide levels are significantly increased ($P < 0.05$) in healthy men compared with women. AUC values for plasma cortisol, GH, and glucagon are significantly increased ($P < 0.01$) in healthy control subjects compared with type 1 diabetic patients.

$789 \pm 260 \text{ } \mu\text{g} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$). GH responses were also increased in healthy men relative to women. GH values were increased approximately twofold ($P < 0.01$) in healthy control subjects compared with type 1 diabetic patients (Fig. 2, Table 2).

Incremental AUC pancreatic polypeptide responses were similar in men and women with type 1 diabetes. However, incremental AUC pancreatic polypeptide responses in healthy control subjects (Fig. 2, Table 2) were increased in men compared with women (21 ± 3 vs. $14 \pm 4 \text{ nmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$). Pancreatic polypeptide responses were similar between type 1 diabetic patients and control subjects.

Direct MSNA. Incremental AUC MSNA (Fig. 1) was increased in type 1 diabetic men compared with women (930 ± 131 vs. 310 ± 248 bursts/min, $P < 0.01$). Incremental AUC MSNA responses were also increased in healthy men compared with women ($1,340 \pm 290$ vs. 528 ± 500 bursts, $P < 0.01$). MSNA responses, overall, were similar between type 1 diabetic patients and healthy control subjects.

Glucose flux. Glucose specific activity (dpm/mg) was in a similar steady state during the control period (Table 3) of each experimental group. By the final 30 min of each insulin infusion, an isotopic steady state existed with a CV of 2.1, 2.3, 2.0, and 2.2% for type 1 diabetic men, diabetic women, healthy men, and healthy women, respectively. EGP was initially similar in all groups ($11 \pm 1 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). By the final 30 min of experiments, EGP in type 1 diabetic men was similar to baseline but was significantly elevated compared with that of type 1 diabetic women (9.4 ± 1.1 vs. $3.9 \pm 1.1 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). EGP in healthy men was also similarly increased compared with that in control women (10.5 ± 0.6 vs. $6.0 \pm 1.7 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). EGP in type 1 diabetic patients did not differ from that in healthy control subjects.

Glucose infusion rates during hypoglycemic clamps were reduced in type 1 diabetic men compared with type 1 diabetic women (2.2 ± 1.1 vs. $7.2 \pm 2.2 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$). No exogenous glucose was required during control studies in men; this was significantly less ($P < 0.01$) than the $4.4 \pm 1.1 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ required during control experiments in women. Tracer-determined glucose utilization rates were similar during the final 30 min of each experimental group (type 1 diabetic men: 12.0 ± 1.6 ; type 1 diabetic women: 10.5 ± 1.6 ; control men: 11.0 ± 0.6 ; and control women: $12.0 \pm 1.6 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Cardiovascular parameters. Heart rate, sBP, dBP, and mean arterial blood pressure were similar at the start of each experiment (Table 4). Heart rate remained at basal levels during hypoglycemia in type 1 diabetic women but increased

TABLE 3
Effects of hyperinsulinemic hypoglycemia on glucose specific activity in overnight-fasted type 1 diabetic and normal humans

	Basal	Final 30 min of clamped hypoglycemia		
		210	225	240
Glucose specific activity (dpm/mmol)				
Type 1 diabetic men	$1,242 \pm 72$	$1,404 \pm 162$	$1,350 \pm 162$	$1,332 \pm 180$
Type 1 diabetic women	$1,498 \pm 148$	$1,674 \pm 180$	$1,674 \pm 180$	$1,620 \pm 198$
Control men	$1,494 \pm 150$	$1,584 \pm 108$	$1,620 \pm 108$	$1,620 \pm 126$
Control women	$1,530 \pm 162$	$1,476 \pm 126$	$1,494 \pm 148$	$1,512 \pm 108$

Data are means \pm SE.

TABLE 4
Effects of hyperinsulinemic hypoglycemia on cardiovascular parameters in overnight-fasted type 1 diabetic and normal humans

	Basal	Final 30 min of clamped hypoglycemia
sBP (mmHg)		
Type 1 diabetic men	113 ± 4	128 ± 5*
Type 1 diabetic women	111 ± 5	110 ± 5
Control men	115 ± 4	130 ± 6†
Control women	104 ± 4	107 ± 6
dBp (mmHg)		
Type 1 diabetic men	65 ± 2	61 ± 3
Type 1 diabetic women	65 ± 4	58 ± 3
Control men	63 ± 2	57 ± 3
Control women	61 ± 2	53 ± 3
Mean arterial pressure (mmHg)		
Type 1 diabetic men	81 ± 3	83 ± 3*
Type 1 diabetic women	81 ± 3	75 ± 4
Control men	81 ± 3	81 ± 3†
Control women	75 ± 3	71 ± 4
Heart rate (beats/min)		
Type 1 diabetic men	65 ± 4	71 ± 3
Type 1 diabetic women	71 ± 4	71 ± 3
Control men	62 ± 4	76 ± 5†
Control women	63 ± 6	69 ± 6

Data are means ± SE. *P < 0.01; type 1 diabetic men values are significantly increased compared with type 1 diabetic women values. †P < 0.05; control men values are significantly increased compared with control women values.

significantly from baseline in type 1 diabetic men (65 ± 3 to 72 ± 3 beats/min, P < 0.05). Heart rate increased significantly during hypoglycemia in normal individuals, with men demonstrating a twofold greater response compared with women (14 ± 2 vs. 6 ± 2 beats/min, P < 0.05). sBP increased similarly during hypoglycemia in type 1 diabetic and normal men (16 ± 2 mmHg, P < 0.01) but remained close to baseline in type 1 diabetic and normal women. dBp fell similarly in all groups (Table 4).

Intermediary metabolism. There was a significant difference (P < 0.01) in blood lactate responses among groups (Table 5). Blood lactate levels were significantly increased by the final 45 min in normal control men (915 ± 95 μmol/l) but remained similar to basal levels in type 1 diabetic patients and control women. Overall, lactate levels during hypoglycemia were significantly lower (P < 0.01) in type 1 diabetic patients compared with normal control subjects. Blood alanine levels remained similar to baseline levels in control men but were significantly suppressed (P < 0.05) in type 1 diabetic patients and control women during hypoglycemia (Table 5). There were also significant differences (P < 0.01) in glycerol metabolism among groups. Blood glycerol levels increased by similar significant (P < 0.01) amounts in type 1 diabetic men and women but remained at basal levels in normal control subjects. NEFAs remained similar to baseline in men and women with type 1 diabetes and were significantly increased compared with large decreases (P < 0.01) occurring during hypoglycemia in normal control subjects (Table 5, Fig. 3). Blood β-hydroxybutyrate increased significantly (approximately

TABLE 5
Effects of hyperinsulinemic hypoglycemia on intermediary metabolites in overnight-fasted type 1 diabetic and normal humans

	Basal	Final 30 min of clamped hypoglycemia
Blood lactate (μmol/l)		
Type 1 diabetic men	870 ± 100	962 ± 120
Type 1 diabetic women	699 ± 82	846 ± 103
Control men	924 ± 95	1,839 ± 128††
Control women	930 ± 118	1,197 ± 150‡
Blood alanine (μmol/l)		
Type 1 diabetic men	283 ± 19	240 ± 18
Type 1 diabetic women	253 ± 25	210 ± 15
Control men	348 ± 30	311 ± 29
Control women	325 ± 38	296 ± 28
Blood glycerol (μmol/l)		
Type 1 diabetic men	32 ± 9	54 ± 10*
Type 1 diabetic women	37 ± 4	59 ± 11*
Control men	32 ± 2	28 ± 4
Control women	52 ± 7	60 ± 6
Blood β-hydroxybutyrate (μmol/l)		
Type 1 diabetic men	40 ± 27	89 ± 41
Type 1 diabetic women	87 ± 42	82 ± 44
Control men	34 ± 11	9 ± 2§
Control women	37 ± 6	11 ± 3§
Plasma NEFA (μmol/l)		
Type 1 diabetic men	402 ± 115	451 ± 108
Type 1 diabetic women	409 ± 71	385 ± 96
Control men	394 ± 63	180 ± 35§
Control women	451 ± 65	211 ± 39§

Data are means ± SE. *P < 0.01; type 1 diabetic men values are significantly increased compared with baseline values. †P < 0.01; control values are significantly increased compared with control women values. ‡P < 0.01; control values are significantly increased compared with type 1 diabetes. §P < 0.01; control values are significantly reduced compared with baseline values.

twofold, P < 0.01) during hypoglycemia in type 1 diabetic men, but it remained similar to baseline in type 1 diabetic women. β-hydroxybutyrate fell significantly (P < 0.01) during hypoglycemia in normal men and women.

Lipid oxidation measured using indirect calorimetry increased to similar levels in type 1 diabetic men and women (Fig. 3). Lipid oxidation in type 1 diabetic patients was over twofold greater (P < 0.01) compared with normal control subjects.

Hypoglycemic symptoms. Hypoglycemic symptoms increased by similar amounts in all groups. Despite significantly reduced sympathetic nervous system (SNS) activity in type 1 diabetic and control women, there was no difference in autonomic symptom responses between men and women. Incremental AUC autonomic symptom scores were: 1,210 ± 150 vs. 1,580 ± 260 for type 1 diabetic men and women and 1,110 ± 280 vs. 1,100 ± 160 for control men and women, respectively.

DISCUSSION

The effect of sex on counterregulatory responses to hypoglycemia in adult type 1 diabetic patients has not been previously defined. This study demonstrates clear sex-related dif-

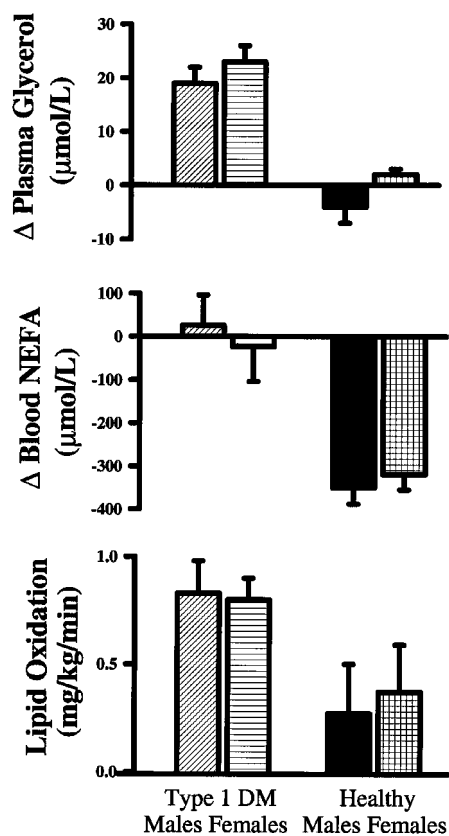


FIG. 3. Effects of peripherally infused insulin ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and hypoglycemia ($3.0 \pm 0.1 \text{ mmol/l}$) on levels of plasma glycerol, blood NEFA, and rates of lipid oxidation in overnight-fasted type 1 diabetic (type 1 DM) and healthy humans. Incremental increases of plasma glycerol, blood NEFA, and rates of lipid oxidation are significantly increased ($P < 0.01$) in type 1 diabetic patients compared with healthy control subjects.

ferences in ANS, neuroendocrine, and metabolic responses to hypoglycemia in type 1 diabetic patients. Despite identical hypoglycemia and insulinemia, plasma EPI, NE, GH, MSNA, EGP, and cardiovascular responses were significantly reduced in type 1 diabetic women.

These present experiments used a glucose clamp technique to control major confounding variables such as differing glycemia and hyperinsulinemia. The hypoglycemic clamp does not measure counterregulation (i.e., the ability of the body to raise plasma glucose toward normal). However, the glucose clamp does allow quantification of counterregulatory mechanisms because of the fact that glycemia can be precisely controlled and equated between groups. In the present experiments, hypoglycemia and insulinemia were equivalent in all groups so that any differences in counterregulatory responses were due to sexual dimorphism. Note that antecedent hypoglycemia was scrupulously avoided by all type 1 diabetic patients for 2 weeks before a study. This was to ensure that measured counterregulatory responses were not affected by additional unquantified hypoglycemia (21,26,27) in any individual.

Long-duration type 1 diabetic patients become totally reliant on SNS and particularly sympathoadrenal (EPI) responses for effective hypoglycemic counterregulation (28). This occurs as a result of the failure of glucagon release in

response to hypoglycemia in these individuals (29). Therefore, the finding that SNS responses were at least twofold greater in type 1 diabetic men compared with women has potential clinical relevance. Increased SNS activity in type 1 diabetic men was demonstrated by elevated circulating catecholamines (EPI and NE) and MSNA. Plasma GH responses were also reduced twofold in type 1 diabetic women compared with men.

The design of this study is unable to identify the mechanism(s) responsible for the sex-related differences in neuroendocrine responses to hypoglycemia in our type 1 diabetic patients. Several potential explanations exist, including differences in body composition, sex hormones, and hypoglycemic thresholds for counterregulatory hormone release. The latter has been suggested by Widom et al. (30). However, subsequent studies by Fanelli et al. (31) and ourselves have found no difference in hypoglycemic thresholds for counterregulatory hormone release in normal men and women. It remains to be determined whether hypoglycemic thresholds differ in men and women with type 1 diabetes.

Other counterregulatory hormones measured in this study (glucagon, cortisol, and pancreatic polypeptide) responded similarly during hypoglycemia in men and women with type 1 diabetes. Glucagon responses to hypoglycemia in both groups of type 1 diabetic individuals were severely blunted, which is characteristic of long-duration type 1 diabetes (32). Pancreatic polypeptide responses in the type 1 diabetic patients were noteworthy for two reasons. First, the magnitude of the response (i.e., not different from normal control subjects) demonstrates that the type 1 diabetic patients involved in the study did not have classical diabetic autonomic neuropathy (33). Second, it is interesting to note that pancreatic polypeptide responses were similar in type 1 diabetic men and women. This differs from the physiology occurring in normal control subjects, where pancreatic polypeptide responses are increased in men.

These present results differ from a report by Arslanian et al. (7) describing hypoglycemic counterregulation in a group of adolescent male and female type 1 diabetic patients. In the study of Arslanian et al., plasma catecholamines, cortisol, glucagon, and pancreatic polypeptide values were similar between sexes despite significantly lower glycemia in men. Plasma GH levels, on the other hand, were reported to be higher in women. Note, however, that there are several significant differences in study design between the present experiments and those of Arslanian et al. The study of Arslanian et al. involved a two-step design whereby individuals underwent an initial 3-h hyperinsulinemic-euglycemic clamp. At the completion of the euglycemic clamp, insulin and glucose infusions were discontinued and neuroendocrine responses to the prevailing drop in glycemia were measured for an additional 60 min. Plasma glucose fell to lower levels in the male compared with the female adolescent subjects, thereby creating differential hypoglycemic signals in the two groups. Furthermore, because of insulin's long-lived cellular effects (21), the previous hyperinsulinemia would likely have had varying confounding effects (suppressive and/or stimulatory) on neuroendocrine hormones (cortisol, pancreatic polypeptide, GH) measured during the subsequent 60-min hypoglycemic period (34–36). Finally, note that Arslanian et al. studied adolescents, and thus it is possible that neuroendocrine counterregulation in that age-

group may be altered compared with counterregulatory responses in adults.

As might be expected, there were some quantitative differences in neuroendocrine responses between type 1 diabetic and control groups. Plasma cortisol, glucagon, and GH responses were reduced in type 1 diabetic patients. Markers of ANS function (EPI, NE, and pancreatic polypeptide) were not blunted in type 1 diabetic patients. This is intriguing, since it is well recognized that intensive therapy is associated with reduced counterregulatory responses to hypoglycemia (15,37). Note that the type 1 diabetic patients involved in this study had reasonably good ($HbA_{1c} \sim 7.7\%$) but not rigorously tight metabolic control ($HbA_{1c} \sim 7.0\%$). It is therefore possible, as previous studies have argued, that an HbA_{1c} in the mid to high 7% range may protect against hypoglycemia-associated autonomic failure (38). However, offsetting this possible benefit must be factored in the increased risk of developing tissue complications of diabetes due to elevated glycemia (8).

Total glucose flux does not increase from baseline during the model of hypoglycemia used in the present study. As a result, little or no exogenous glucose was infused during the present experiments. Therefore, glucose tracer (tritiated glucose) could not be delivered via an exogenous glucose infusate and had to be administered by a constant infusion. However, by the final 45 min of each experiment, tracer, tracee, and, consequently, glucose specific activity were in steady state. These conditions, as demonstrated by Norwich (39), allow reliable estimates of whole-body glucose kinetics to be obtained. EGP was reduced approximately twofold in type 1 diabetic and control women compared with men. This finding is consistent with previous data demonstrating a greater reduction of EGP in women during a 64-h fast compared with men (40). The reduced EGP occurring in women during this present study is most plausibly explained by the commensurately lowered neuroendocrine responses to hypoglycemia.

Lipolysis is an important counterregulatory mechanism (41). Previous studies have demonstrated that lipolysis may contribute up to 25% of total glucose counterregulation during prolonged hypoglycemia in normal subjects (42). During hypoglycemia, glycerol is a significant substrate for gluconeogenesis (43). NEFA oxidation provides energy for increased gluconeogenesis, and preferential NEFA oxidation by skeletal muscle limits glucose utilization. Metabolic data from the present study indicate that lipolysis may be even more important during hypoglycemia in type 1 diabetic patients compared with normal control subjects.

Plasma NEFA levels were significantly increased in type 1 diabetic patients compared with normal subjects. These increased NEFA levels could have been due to impaired reesterification or increased lipolysis. However, blood glycerol levels also increased substantially in type 1 diabetic patients. Therefore, since previous studies have demonstrated that increases in peripheral glycerol levels correlate very highly with isotopically measured elevations in rates of glycerol appearance (32), we believe, despite equivalent hypoglycemia, insulinemia, and sympathetic drive in both groups, that there was a relative increase in lipolysis in the type 1 diabetic patients compared with the normal control subjects.

Indirect calorimetry data from this study demonstrate that greater lipid oxidation occurred during hypoglycemia in type 1 diabetic patients compared with normal control sub-

jects. The site of increased lipid oxidation (i.e., liver or muscle) could not be directly identified. However, levels of the ketone body, β -hydroxybutyrate, were also significantly increased in type 1 diabetic patients compared with normal control subjects. This finding is consistent with an increased delivery to and oxidation of NEFAs by the liver. It would therefore appear that elevated lipolytic responses lead to increased lipid oxidation (presumably liver) during hypoglycemia in type 1 diabetic patients. The mechanism(s) responsible for increased lipolytic responses during hypoglycemia in type 1 diabetes is/are not fully elucidated. Possibilities include enhanced responsiveness to either EPI or NE and/or direct sympathetic drive, or even a combination of both. Information isolating enhanced responsiveness to EPI as the putative mechanism for increased lipolytic responses is somewhat conflicting. Divertie et al. (44) have demonstrated no difference in lipolytic responsiveness to EPI in type 1 diabetic patients compared with normal individuals under euglycemic conditions. Bolinder et al. (45), on the other hand, have reported that the increased lipolytic responses occurring during hypoglycemia in type 1 diabetic patients may be due to enhanced β -adrenoreceptor sensitivity to EPI.

Despite significantly increased SNS activity in men, autonomic hypoglycemic symptom responses, NEFA levels, and lipid oxidation were similar in men and women. These results appear to indicate that women may have relatively increased sensitivity to SNS drive for autonomic symptom awareness and lipolysis during hypoglycemia. Interestingly, cardiovascular responses were reduced in women relative to men during hypoglycemia and therefore proportional to prevailing SNS drive. Clinically, these results appear to have potential relevance. It is possible that women with type 1 diabetes may be able to partially overcome reduced SNS activity during hypoglycemia by increased autonomic symptom awareness and lipolytic sensitivity.

In summary, these studies demonstrate that compared with men, women with type 1 diabetes have markedly reduced neuroendocrine, ANS, and cardiovascular responses to clamped moderate hypoglycemia. Major counterregulatory mechanisms (SNS activity and EGP) were reduced by 50% in type 1 diabetic women compared with men. We conclude that adult type 1 diabetic women have clearly reduced counterregulatory responses to hypoglycemia compared with type 1 diabetic men. Further studies are required to unravel the clinical conundrum of why the incidence of severe hypoglycemia appears to be equivalent in type 1 diabetic men and women despite markedly reduced counterregulatory responses in the latter.

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