Limited Impact of Vigorous Exercise on Defenses Against Hypoglycemia

Relevance to Hypoglycemia-Associated Autonomic Failure

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Hypoglycemia-associated autonomic failure (HAAF)—reduced autonomic (including adrenomedullary epinephrine) and symptomatic responses to hypoglycemia caused by recent antecedent hypoglycemia—plays a key role in the pathogenesis of defective glucose counterregulation and hypoglycemia unawareness and thus iatrogenic hypoglycemia in type 1 diabetes. On the basis of the findings that cortisol infusion mimics and deficient or inhibited cortisol secretion minimizes this phenomenon, it has been suggested that the cortisol response to antecedent hypoglycemia mediates HAAF. We tested the hypothesis that any stimulus that releases cortisol, such as exercise, reduces autonomic and symptomatic responses to subsequent hypoglycemia. Thirteen healthy young adults (four women) were studied on three occasions in random sequence: *1*) cycle exercise (~70% peak oxygen consumption) from 0830 to 0930 h and from 1200 to 1300 h on day 1 and hyperinsulinemic ($2.0\ mU \cdot kg^{-1} \cdot min^{-1}$) stepped hypoglycemic (85, 75, 65, 55, and 45 mg/dl) clamps on day 2, *2*) rest on day 1 and identical hypoglycemic clamps on day 2, and *3*) hyperinsulinemic-euglycemic clamps. Exercise raised plasma cortisol concentrations to 16.9 ± 1.9 (0930 h) and 16.6 ± 1.6 μg/dl (1300 h) on day 1. Compared with rest on day 1, exercise on day 1 was associated with reduced epinephrine ($P = 0.0113$) responses—but not norepinephrine ($P = 0.6270$), neurogenic symptom ($P = 0.6470$), pancreatic polypeptide ($P = 0.0629$), or glucagon ($P = 0.0436$, but higher) responses—to hypoglycemia on day 2. However, the effect was small. (The final day 2 hypoglycemia epinephrine values were 765 ± 106 pg/ml after rest on day 1 and 550 ± 94 pg/ml after exercise on day 1 compared with 30 ± 6 pg/ml during euglycemia.) These data are consistent with the hypothesis that the cortisol response to hypoglycemia mediates in part the reduced epinephrine response to subsequent hypoglycemia, one key component of HAAF in type 1 diabetes. However, the small effect suggests that an additional factor or factors may well be involved. These data do not support the hypothesis that the cortisol response to hypoglycemia mediates the reduced neurogenic symptom response to subsequent hypoglycemia, another key component of HAAF in type 1 diabetes. *Diabetes* **51:1485–1492, 2002**

Iatrogenic hypoglycemia is the limiting factor, both conceptually and in practice, in the glycemic management of diabetes (1–4). At least in type 1 diabetes, iatrogenic hypoglycemia is the result of the interplay of relative or absolute therapeutic insulin excess and compromised physiological and behavioral defenses against developing hypoglycemia (1,2,5–9). The concept of hypoglycemia-associated autonomic failure (HAAF) in type 1 diabetes (10–16) posits that recent antecedent iatrogenic hypoglycemia, by reducing the autonomic (including the adrenomedullary epinephrine as well as the sympathetic neural norepinephrine and acetylcholine) responses and the resultant neurogenic symptomatic responses to a given level of subsequent hypoglycemia, causes the clinical syndrome of hypoglycemia unawareness and—by reducing epinephrine responses in the setting of absent glucagon responses—the clinical syndrome of defective glucose counterregulation.

The mechanism of HAAF is unknown. It has been suggested that recent antecedent hypoglycemia increases brain glucose uptake during subsequent hypoglycemia (17,18), but we found no effect of recent antecedent hypoglycemia on blood-to-brain glucose transport, cerebral glucose metabolism, or cerebral blood flow (19). The mediator of HAAF is also unknown. On the basis of the findings that antecedent cortisol infusion mimics the phenomenon (20) and that deficient (21) or metyrapone-inhibited (22) cortisol secretion minimizes the phenomenon, it has been suggested that the cortisol response to antecedent hypoglycemia mediates the reduced responses to subsequent hypoglycemia. If cortisol is the mediator of HAAF, then any stimulus that releases cortisol, such as exercise, should reduce the responses to subsequent hypoglycemia. Indeed, two bouts (morning and afternoon) of relatively mild exercise (50% maximum oxygen consumption × 90 min), compared with rest, has been reported to reduce the epinephrine, norepinephrine, muscle sympathetic nerve activity, pancreatic polypeptide, glucagon, and growth hormone responses—but not the symptomatic or cortisol responses—to hypoglycemia the next day (23).

We tested the hypothesis that vigorous exercise reduces autonomic (including adrenomedullary epinephrine) and symptomatic responses to hypoglycemia the following day, specifically that it shifts the glycemic thresholds for

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HAAF, hypoglycemia-associated autonomic failure; $V_{O_{2peak}}$, peak oxygen consumption.

these responses to lower plasma glucose concentrations. To do so we applied the hyperinsulinemic stepped hypoglycemic clamp technique (24) to healthy young adults following two bouts of vigorous exercise or rest the previous day.

RESEARCH DESIGN AND METHODS

Subjects. Thirteen healthy young adults gave their informed consent to participate in this study, which was approved by the Washington University Human Studies Committee and conducted at the Washington University General Clinical Research Center (GCRC). Four were women; nine were men. Their mean (±SD) age was 23.3 ± 2.4 years. Their mean BMI was 23.3 ± 3.8 kg/m^2.

Experimental design. Subjects were studied on three separate occasions in random sequence: *1*) cycle exercise at 70% peak oxygen consumption ($V\text{O}_{2\text{peak}}$) from 0830 to 0930 h and from 1200 to 1300 h on day 1 and hyperinsulinemic (2.0 mU · kg^{-1} · min^{-1}; 12 pmol · kg^{-1} · min^{-1}) stepped hypoglycemic clamps (hourly steps at ~85, 75, 65, 55, and 45 mg/dl; 4.7, 4.2, 3.6, 3.1, and 2.5 mmol/l) (24) the morning of day 2, *2*) rest on day 1 and identical hyperinsulinemic stepped hypoglycemic clamps on day 2- and *3*) hyperinsulinemic euglycemic (~90 mg/dl, 5.0 mmol/l) clamps.

Before entry into the study, all potential subjects were screened to ensure that they met the inclusion criteria—good health on the basis of a medical history and physical examination, normal hematocrits, fasting plasma glucose concentrations, and electrocardiograms—and $V\text{O}_{2\text{peak}}$ was determined as described previously (25). On the exercise and rest days (day 1), the subjects reported to the GCRC at ~0730 h, a line was inserted into an antecubital vein (for sampling), and they either exercised on a cycle ergometer at ~70% $V\text{O}_{2\text{peak}}$ from 0830 to 0930 h and again from 1200 to 1300 h or rested in the sitting position. Blood samples were obtained at 30-min intervals from 0830 through 1030 h and from 1200 through 1400 h. A snack was provided at 1100 h. On the next day (day 2), the subjects reported to the GCRC after an overnight fast at ~0630 h. An intravenous line (for insulin and glucose infusions) and a line in a hand vein (with that hand kept in an ~60°C plexiglas box for arterialized venous blood sampling) were inserted, and electrocardiogram leads and a vital signs monitor (Propaq Encore; Protocol Systems, Beverton, OR) were attached. The subjects remained supine throughout the study. After 30 min of supine rest and starting at ~0730 h, regular insulin was infused in a dose of 2.0 mU · kg^{-1} · min^{-1} (12.0 pmol · kg^{-1} · min^{-1}) from 0 through 300 min. Glucose (20%) was infused at variable rates, based on plasma glucose measurements with a glucose oxidase method (Yellow Springs Analyzer 2; Yellow Springs Instruments, Yellow Springs, OH) every 5 min, to maintain plasma glucose concentrations at target levels of 85, 75, 65, 55, and 45 mg/dl (4.7, 4.2, 3.6, 3.1, and 2.5 mmol/l) in hourly steps (24). The euglycemic clamps, on a separate occasion, were identical except that plasma glucose concentrations were held at ~90 mg/dl (5.0 mmol/l). Arterialized venous samples for analytes (listed below) other than glucose and symptom scores were obtained at 30-min intervals throughout. Heart rates and blood pressures were recorded at 15-min intervals; the electrocardiogram was monitored throughout.

Analytical methods. Plasma insulin (26), C-peptide (26), glucagon (27), pancreatic polypeptide (28), growth hormone (29), and cortisol (30) were measured with radioimmunoassays. Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (31). Serum nonesterified fatty acids (32) and blood β-hydroxybutyrate (33), lactate (34), and alanine (35) were measured with enzymatic methods. Symptoms of hypoglycemia were quantified by asking the subjects to score (0 [none] to 6 [severe]) each of 12 symptoms: six neurogenic symptoms (adrenergic: heart pounding, shaky/tremulous, and nervous/anxious; cholinergic: sweaty, hungry, and tingling) and six neuroglycopenic symptoms (difficulty thinking/confused, tired/drowsy, weak, warm, faint, and dizzy) based on our published data (36).

Statistical methods. Data in this manuscript are reported as the means ± SE except where the SD is specified. Data were analyzed by general linear model repeated measures ANOVA. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Exercise and rest (day 1). Cycle exercise targeted at 70% $V\text{O}_{2\text{peak}}$ (37 ± 6 [SD] ml · kg^{-1} · min^{-1}) raised oxygen consumption to 69 ± 1% $V\text{O}_{2\text{peak}}$ from 0830 to 0930 h and to 67 ± 2% $V\text{O}_{2\text{peak}}$ from 1200 to 1300 h on the exercise day 1. As shown in Fig. 1, it raised plasma cortisol concentrations to 16.5 ± 2.0 μg/dl (455 ± 55 nmol/l) compared with

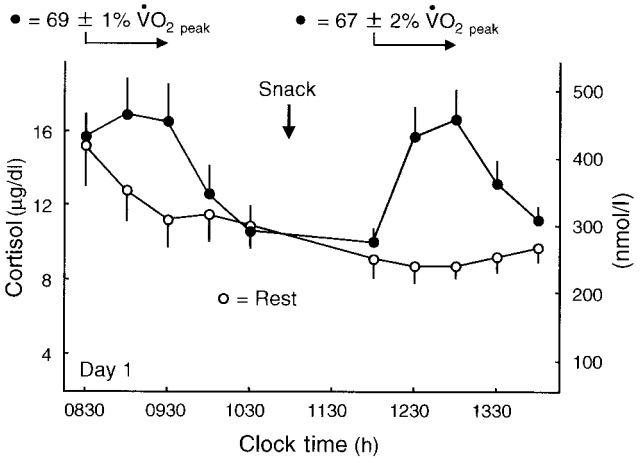

FIG. 1. Mean ± SE plasma cortisol concentrations before, during, and after vigorous exercise on day 1 and before, during, and after rest on day 1 in 13 healthy subjects.

11.2 ± 1.5 μg/dl (310 ± 40 nmol/l) at the same time on the rest day 1 and to 16.6 ± 1.6 μg/dl (460 ± 45 nmol/l) compared with 8.7 ± 0.7 μg/dl (240 ± 20 nmol/l) at the same time on the rest day 1 at 0930 h and 1300 h, respectively.

Hyperinsulinemic stepped hypoglycemic clamps (day 2) and euglycemic clamps. Target plasma glucose concentrations were achieved during the hyperinsulinemic stepped hypoglycemic and euglycemic clamps (Fig. 2). Plasma insulin concentrations were comparable (P = 0.2252), ~100 μU/ml (600 pmol/l), during all three hyperinsulinemic clamps (Fig. 3). Plasma C-peptide concentrations declined, from 1.7 ± 0.3 ng/ml (0.6 ± 0.1 nmol/l) to 1.1 ± 0.1 ng/ml (0.4 ± 0.0 nmol/l), during hyperinsulinemic euglycemia and to a greater extent ($P < 0.0001$) during hyperinsulinemic hypoglycemia, to 0.3 ± 0.1 ng/ml (0.1 ± 0.0 nmol/l) and 0.1 ± 0.0 ng/ml (<0.1 ± 0.0 nmol/l), on the days after exercise and after rest, respectively (Fig. 3).

The plasma epinephrine response ($P < 0.0001$) to hyperinsulinemic hypoglycemia was reduced slightly but

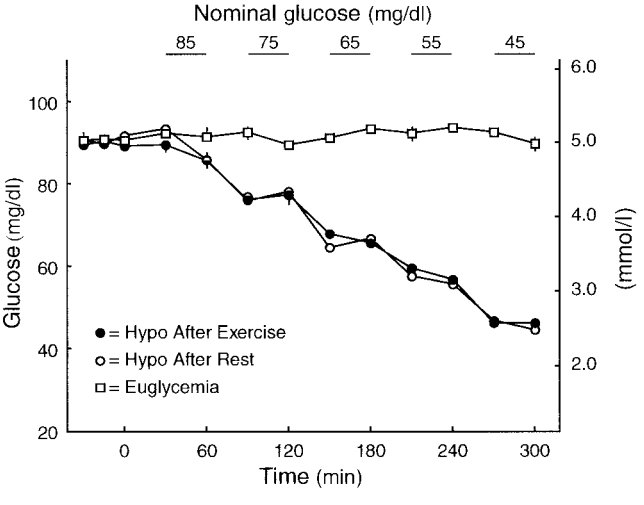

FIG. 2. Mean ± SE plasma glucose concentrations during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects.

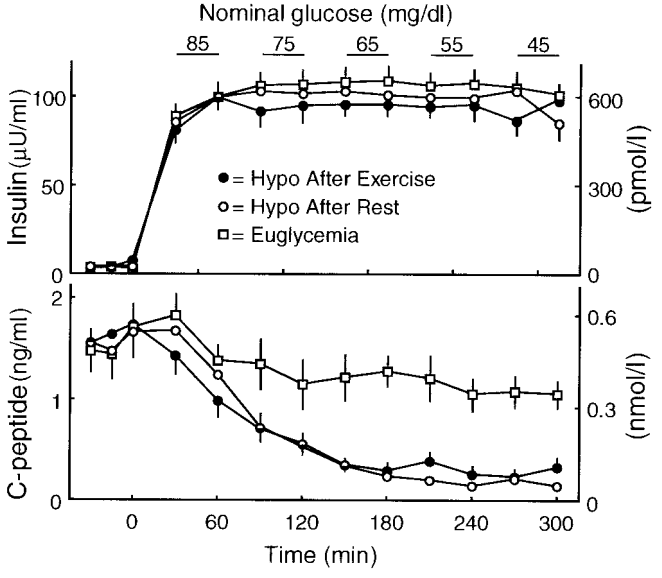

FIG. 3. Mean ± SE plasma insulin and C-peptide concentrations during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects.

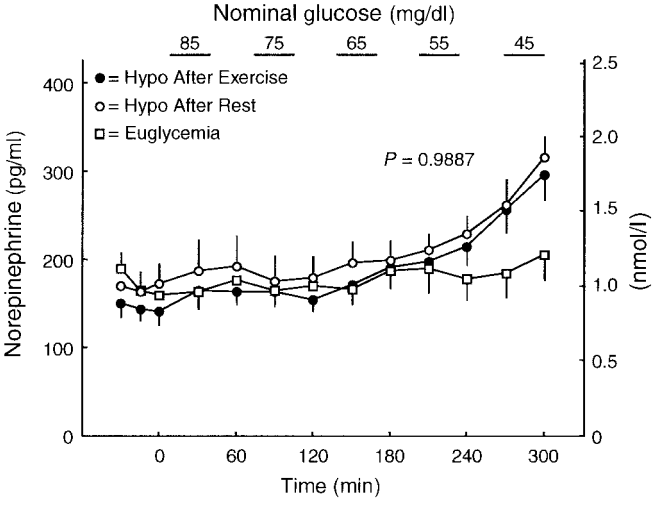

FIG. 5. Mean ± SE plasma norepinephrine concentrations during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

significantly ($P = 0.0113$) on the day after exercise compared with the day after rest (Fig. 4). The final values (300 min, glucose ~45 mg/dl) were 550 ± 94 pg/ml (3,000 ± 510 pmol/l) on the day after exercise and 765 ± 106 pg/ml (4,180 ± 580 pmol/l) on the day after rest compared with a final value (300 min, glucose ~90 mg/dl) of 30 ± 6 pg/ml (160 ± 30 pmol/l) during hyperinsulinemic euglycemia.

The plasma norepinephrine response ($P = 0.0003$) to hyperinsulinemic hypoglycemia was unaltered ($P = 0.6270$) by exercise on the previous day (Fig. 5). The final values (300 min, glucose ~45 mg/dl) were 296 ± 28 pg/ml (1.75 ± 0.17 nmol/l) on the day after exercise and 315 ± 26 pg/ml (1.86 ± 0.15 nmol/l) on the day after rest compared with a final value (300 min, glucose ~90 mg/dl) of 205 ± 28 pg/ml (1.21 ± 0.17 nmol/l) during hyperinsulinemic euglycemia.

The neurogenic (Fig. 6) and neuroglycopenic (Fig. 7) symptom responses ($P = 0.0009$ and <0.0001, respectively) to hyperinsulinemic hypoglycemia were also unaltered ($P = 0.6470$ and 0.6624, respectively) by exercise on the previous day. The final (300 min, glucose ~45 mg/dl) neurogenic symptom scores were 8.6 ± 2.1 on the day after exercise and 8.3 ± 1.6 on the day after rest compared with a final score (300 min, glucose ~90 mg/dl) of 2.9 ± 0.6 during hyperinsulinemic euglycemia. The final (300 min, glucose ~45 mg/dl) neuroglycopenic symptom scores were 6.5 ± 1.8 on the day after exercise and 6.2 ± 1.9 on the day after rest compared with a final score (300 min, glucose ~90 mg/dl) of 2.2 ± 1.0 during hyperinsulinemic euglycemia.

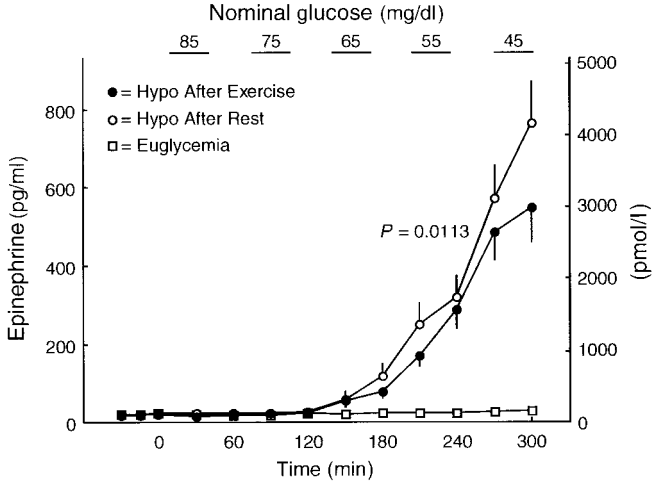

FIG. 4. Mean ± SE plasma epinephrine concentrations during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

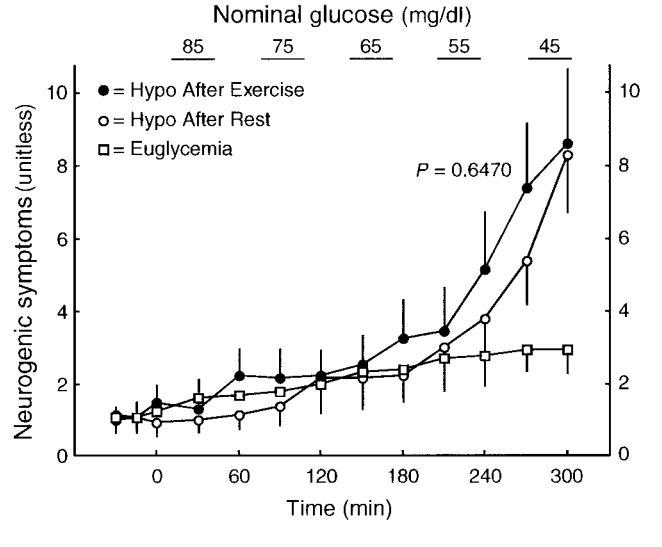

FIG. 6. Mean ± SE neurogenic (autonomic) symptom scores during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

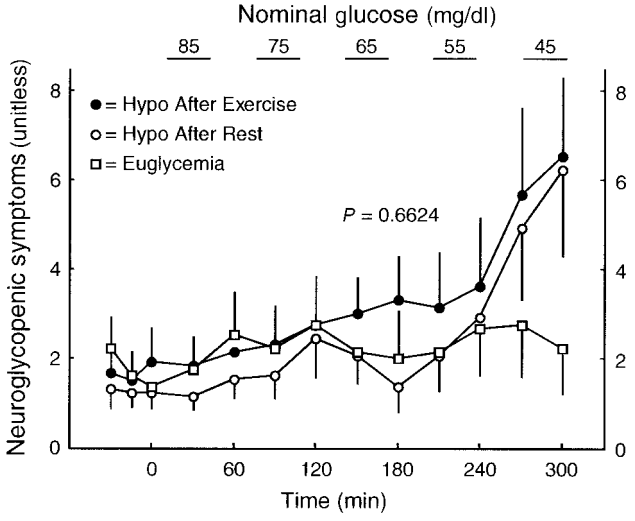

FIG. 7. Mean ± SE neuroglycopenic symptom scores during hyperinsulinemic stepped hypoglycemia (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

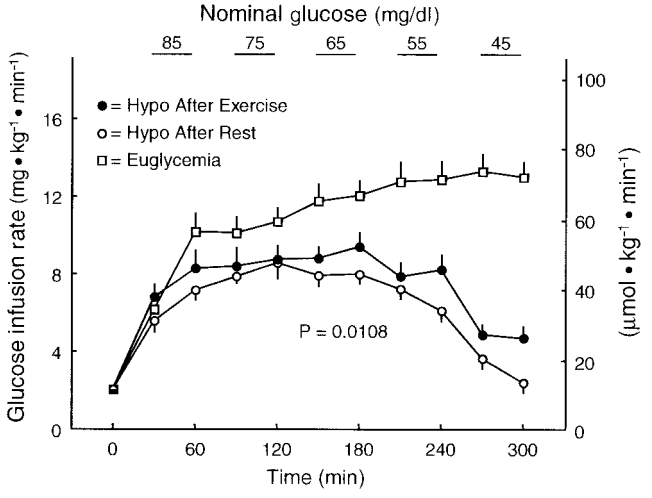

FIG. 9. Mean ± SE glucose infusion rates during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

The plasma glucagon response ($P < 0.0001$) to hyperinsulinemic hypoglycemia was not reduced by exercise on the previous day (Fig. 8); indeed, the glucagon levels were slightly higher ($P = 0.0436$) on the day after exercise. The final values (300 min, glucose ~45 mg/dl) were 88 ± 7 pg/ml (25 ± 2 pmol/l) on the day after exercise and 80 ± 8 pg/ml (23 ± 2 pmol/l) on the day after rest compared with a final value (300 min, glucose ~90 mg/dl) of 37 ± 2 pg/ml (11 ± 1 pmol/l) during hyperinsulinemic euglycemia.

The glucose infusion rates required to maintain the plasma glucose steps during hyperinsulinemic hypoglycemia were slightly but significantly ($P = 0.0108$) higher on the day after exercise compared with the day after rest (Fig. 9). The final values (300 min, glucose ~45 mg/dl) were 4.7 ± 0.6 mg · kg^{-1} · min^{-1} (26 ± 3 μmol · kg^{-1} · min^{-1}) on the day after exercise and 2.4 ± 0.6 mg · kg^{-1} · min^{-1} (13 ± 3 μmol · kg^{-1} · min^{-1}) on the day after rest compared with a final value (300 min, glucose ~90 mg/dl) of 13.0 ± 0.8 mg · kg^{-1} · min^{-1} (72 ± 4 μmol · kg^{-1} · min^{-1}) during hyperinsulinemic euglycemia.

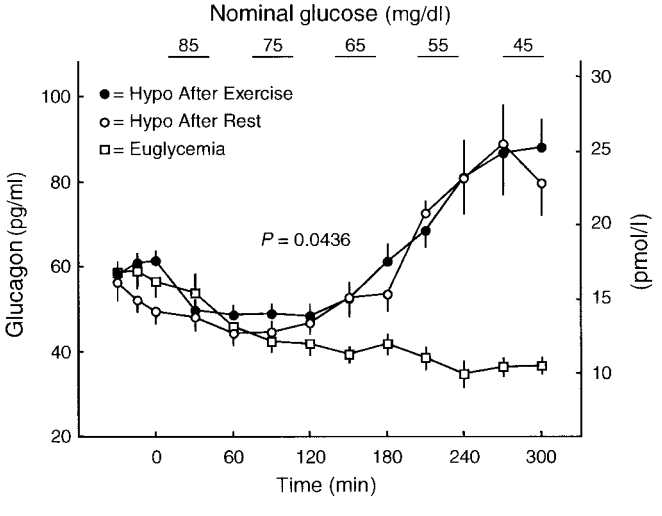

FIG. 8. Mean ± SE plasma glucagon concentrations during hyperinsulinemic stepped hypoglycemic (Hypo) clamps on day 2 after vigorous exercise on day 1 (●) and after rest on day 1 (○) and during hyperinsulinemic-euglycemic (Euglycemia) clamps (□) in 13 healthy subjects. The *P* value shown refers to the hypoglycemia contrast.

The plasma growth hormone response ($P < 0.0001$) but not the plasma pancreatic polypeptide or cortisol response both ($P < 0.0001$) to hypoglycemia was reduced significantly on the day after exercise compared with the day after rest (Table 1). The *P* values were 0.0090 for growth hormone, 0.0629 for pancreatic polypeptide, and 0.1275 for cortisol. There were no sex differences. However, only four women were studied.

Serum nonesterified fatty acid ($P = 0.3900$) and blood β-hydroxybutyrate ($P = 0.8465$) concentrations were suppressed comparably under all three study conditions (Table 2). Increments in blood lactate levels were similar (0.3064) during hypoglycemia on the days after exercise and rest (Table 2). Similarly, there was no difference in blood alanine levels ($P = 0.1921$).

Heart rates ($P = 0.0788$) and systolic blood pressures ($P = 0.3304$) were similar under all three study conditions (Table 3). Diastolic blood pressures declined ($P = 0.0074$) during the hypoglycemic clamps, but there was no difference after exercise compared with after rest ($P = 0.6678$).

DISCUSSION

These data demonstrate that two bouts of vigorous cycle exercise—69 ± 1% and 67 ± 2% of peak oxygen consumption from 0830 to 0930 h and from 1200 to 1300 h, respectively—raised plasma cortisol concentrations during exercise and reduced the plasma epinephrine and growth hormone responses to hyperinsulinemic stepped hypoglycemia on the next day slightly but significantly. Plasma norepinephrine, neurogenic and neuroglycopenic symptom and plasma pancreatic polypeptide, glucagon, and cortisol responses to hypoglycemia were not reduced by exercise on the previous day.

These findings differ quantitatively and in many respects qualitatively, from those of Galassetti et al. (23), who assessed the impact of two somewhat longer bouts of moderate exercise (~50% Vo_{2peak} for 90 min) on responses

TABLE 1
Plasma pancreatic polypeptide, cortisol, and growth hormone concentrations during hyperinsulinemic-hypoglycemic clamps on day 2 after exercise (Ex/Hypo) and after rest (Rest/Hypo) on day 1 and during hyperinsulinemic-euglycemic clamps (Euglycemia)*

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300
Pancreatic polypeptide (pg/ml)													
Ex/Hypo†	68 ± 19	62 ± 17	96 ± 48	55 ± 14	78 ± 18	65 ± 13	67 ± 14	50 ± 13	56 ± 11	95 ± 25	307 ± 66	357 ± 69	479 ± 62
Rest/Hypo	72 ± 7	75 ± 7	75 ± 5	70 ± 5	62 ± 4	60 ± 3	57 ± 4	62 ± 5	108 ± 44	253 ± 73	363 ± 73	575 ± 62	521 ± 68
Euglycemia	68 ± 15	67 ± 9	63 ± 8	52 ± 7	55 ± 6	41 ± 5	48 ± 5	56 ± 6	44 ± 5	53 ± 7	47 ± 6	56 ± 7	54 ± 7
Cortisol (μg/dl)													
Ex/Hypo‡	13.4 ± 1.0	12.3 ± 1.3	12.0 ± 1.4	10.1 ± 1.1	10.2 ± 0.9	9.1 ± 0.8	7.6 ± 0.5	7.6 ± 0.6	9.8 ± 1.2	10.1 ± 1.2	12.9 ± 1.1	15.1 ± 1.4	207 ± 1.6
Rest/Hypo	12.4 ± 1.0	12.3 ± 0.9	13.7 ± 2.2	11.9 ± 2.4	11.4 ± 1.7	11.5 ± 1.1	12.0 ± 1.7	11.6 ± 2.0	10.8 ± 1.1	12.7 ± 1.4	15.2 ± 1.6	20.7 ± 2.5	23.2 ± 1.8
Euglycemia	18.8 ± 2.7	18.9 ± 2.7	17.3 ± 2.5	15.5 ± 2.9	15.8 ± 2.6	12.8 ± 2.8	10.6 ± 2.0	10.0 ± 1.6	10.9 ± 1.6	11.6 ± 1.3	9.7 ± 1.4	9.2 ± 1.4	9.7 ± 1.3
Growth hormone (ng/ml)													
Ex/Hypo§	2.8 ± 1.4	3.6 ± 1.6	3.2 ± 1.4	3.3 ± 1.3	5.0 ± 2.4	2.6 ± 1.4	2.5 ± 1.2	4.1 ± 1.5	3.6 ± 1.4	5.2 ± 1.8	11.7 ± 2.7	13.8 ± 2.5	20.4 ± 2.0
Rest/Hypo	1.9 ± 0.6	1.8 ± 0.8	2.6 ± 1.3	1.5 ± 0.7	0.9 ± 0.4	0.6 ± 0.0	0.5 ± 0.0	1.0 ± 0.5	4.4 ± 1.4	11.0 ± 3.3	18.2 ± 3.4	20.7 ± 4.2	25.5 ± 3.3
Euglycemia	3.3 ± 1.3	3.6 ± 1.8	2.7 ± 1.4	2.0 ± 1.0	2.6 ± 1.2	2.3 ± 0.8	2.5 ± 1.4	1.1 ± 0.5	1.1 ± 0.4	1.2 ± 0.6	1.9 ± 0.9	3.0 ± 1.4	2.5 ± 1.4

Data are means ± SE. *To convert pancreatic polypeptide to pmol/l, multiply by 0.239; cortisol to nmol/l, multiply by 27.59; and growth hormone to pmol/l, multiply by 44.15. †$P = 0.0629$ vs. Rest/Hypo; ‡$P = 0.1275$ vs. Rest/Hypo; §$P = 0.0090$ vs. Rest/Hypo.

TABLE 2
Serum nonesterified fatty acid and blood β-hydroxybutyrate, lactate, and alanine concentrations during hyperinsulinemic-hypoglycemic clamps on day 2 after exercise (Ex/Hypo) and after rest (Rest/Hypo) on day 1 and during hyperinsulinemic-euglycemic clamps (Euglycemia)

	Time (min)												
	−30	−15	0	30	60	90	120	150	180	210	240	270	300
Nonesterified fatty acids (μmol/l)													
Ex/Hypo	511 ± 63	455 ± 55	456 ± 52	204 ± 32	87 ± 15	122 ± 39	64 ± 19	43 ± 5	48 ± 5	42 ± 6	49 ± 8	63 ± 16	70 ± 18
Rest/Hypo	323 ± 35	367 ± 38	376 ± 47	163 ± 34	93 ± 17	69 ± 7	61 ± 7	83 ± 28	50 ± 5	47 ± 6	57 ± 7	60 ± 8	78 ± 13
Euglycemia	280 ± 38	320 ± 37	341 ± 41	131 ± 19	80 ± 6	68 ± 5	66 ± 6	56 ± 6	48 ± 4	57 ± 6	51 ± 5	103 ± 38	49 ± 5
β-Hydroxybutyrate (μmol/l)													
Ex/Hypo	181 ± 41	173 ± 47	174 ± 40	104 ± 15	46 ± 11	52 ± 12	45 ± 13	28 ± 7	61 ± 12	53 ± 11	48 ± 9	46 ± 8	39 ± 9
Rest/Hypo	87 ± 10	96 ± 9	84 ± 8	72 ± 9	58 ± 7	54 ± 8	41 ± 8	45 ± 7	49 ± 7	47 ± 8	50 ± 14	43 ± 7	48 ± 9
Euglycemia	159 ± 72	146 ± 55	114 ± 44	87 ± 22	59 ± 11	55 ± 8	56 ± 12	49 ± 10	47 ± 7	52 ± 10	44 ± 9	48 ± 9	58 ± 12
Lactate (μmol/l)													
Ex/Hypo	766 ± 107	684 ± 80	774 ± 94	801 ± 79	1245 ± 101	1267 ± 86	1216 ± 95	1218 ± 113	1362 ± 101	1456 ± 140	1655 ± 120	1796 ± 128	2043 ± 170
Rest/Hypo	980 ± 83	1011 ± 113	890 ± 101	1025 ± 110	1400 ± 94	1364 ± 109	1215 ± 66	1276 ± 105	1346 ± 98	1539 ± 151	1568 ± 116	1770 ± 152	2066 ± 180
Euglycemia	985 ± 109	943 ± 85	850 ± 87	1050 ± 83	1367 ± 89	1444 ± 127	1306 ± 99	1282 ± 96	1180 ± 71	1261 ± 97	1402 ± 103	1316 ± 109	1369 ± 116
Alanine (μmol/l)													
Ex/Hypo	401 ± 86	343 ± 52	419 ± 63	358 ± 47	383 ± 55	352 ± 58	348 ± 74	332 ± 57	370 ± 43	384 ± 69	349 ± 53	311 ± 47	297 ± 49
Rest/Hypo	500 ± 122	516 ± 129	498 ± 126	425 ± 113	450 ± 125	430 ± 96	390 ± 104	422 ± 98	394 ± 85	377 ± 81	354 ± 82	407 ± 103	372 ± 87
Euglycemia	481 ± 87	479 ± 86	474 ± 78	493 ± 91	458 ± 87	455 ± 98	380 ± 77	335 ± 70	323 ± 46	354 ± 66	359 ± 77	345 ± 53	371 ± 70

Data are means ± SE.

TABLE 3
Heart rate, systolic blood pressure, and diastolic blood pressure during hyperinsulinemic-hypoglycemic clamps on day 2 after exercise (Ex/Hypo) and after rest (Rest/Hypo) on day 1 and during hyperinsulinemic-euglycemic clamps (Euglycemia)

	Time (min)											
	−30	−15	0	30	60	90	120	150	180	210	240	270	300
Heart rate (bpm)													
Ex/Hypo	67 ± 3	65 ± 3	65 ± 2	70 ± 2	71 ± 3	70 ± 3	72 ± 3	72 ± 3	731 ± 3	71 ± 3	76 ± 3	78 ± 3	72 ± 3
Rest/Hypo	65 ± 2	64 ± 3	62 ± 3	64 ± 3	61 ± 2	67 ± 3	68 ± 4	69 ± 3	70 ± 3	74 ± 3	75 ± 3	76 ± 4	74 ± 3
Euglycemia	63 ± 30	64 ± 3	67 ± 3	64 ± 2	70 ± 3	69 ± 3	67 ± 2	70 ± 3	73 ± 3	73 ± 4	73 ± 3	73 ± 3	72 ± 4
Systolic blood pressure (mmHg)													
Ex/Hypo	115 ± 30	112 ± 3	114 ± 3	113 ± 4	117 ± 4	113 ± 4	116 ± 4	114 ± 4	116 ± 4	115 ± 4	114 ± 3	111 ± 4	114 ± 5
Rest/Hypo	118 ± 4	116 ± 3	116 ± 3	116 ± 4	123 ± 6	123 ± 4	119 ± 3	118 ± 3	118 ± 4	119 ± 4	118 ± 4	114 ± 5	119 ± 5
Euglycemia	116 ± 4	116 ± 3	115 ± 3	115 ± 4	117 ± 3	118 ± 4	116 ± 4	112 ± 5	116 ± 5	119 ± 5	119 ± 4	117 ± 4	123 ± 5
Diastolic blood pressure (mmHg)													
Ex/Hypo	60 ± 2	58 ± 3	60 ± 3	57 ± 3	56 ± 3	55 ± 4	54 ± 3	54 ± 3	55 ± 3	52 ± 2	49 ± 2	48 ± 3	48 ± 2
Rest/Hypo	60 ± 3	60 ± 3	60 ± 3	60 ± 3	57 ± 2	58 ± 3	56 ± 3	53 ± 2	55 ± 2	51 ± 2	53 ± 2	49 ± 2	52 ± 2
Euglycemia	62 ± 2	61 ± 3	60 ± 2	60 ± 3	57 ± 2	58 ± 2	55 ± 3	55 ± 3	57 ± 2	57 ± 2	58 ± 3	56 ± 3	61 ± 4

Data are means ± SE.

to hyperinsulinemic hypoglycemia (54 mg/dl, 3.0 mmol/l) on the next day. First, in the present study, the impact of previous exercise on the epinephrine and growth hormone responses to subsequent hypoglycemia was small. Clearly, these responses were not eliminated; the data suggest a small shift of the glycemic thresholds for these responses to slightly lower plasma glucose concentrations. Second, in contrast to the findings of Galassetti et al. (23), the present data show no effect of previous vigorous exercise on the plasma norepinephrine, pancreatic polypeptide, and glucagon responses to subsequent hypoglycemia. The reasons for these differences are not entirely clear. The day 1 exercise patterns differed. Ours was more vigorous, theirs was somewhat longer. Nonetheless, on balance it would seem that the exercise stimuli were similar. Our final hypoglycemic step was shorter than their hypoglycemic clamp, but our two steps, at 55 and 45 mg/dl over 2 h, would have produced a comparable if not more potent hypoglycemic stimulus. Our subjects were somewhat more fit. Their exercise protocol resulted in higher absolute plasma cortisol concentrations. We suspect that this is an important difference. It may be relevant that the control (rest) data in the present study were obtained in the same subjects studied concurrently in random sequence with the exercise study and under identical conditions aside from rest or exercise on day 1.

Despite these seemingly substantive quantitative differences, there is some qualitative agreement with respect to the impact of previous exercise on the autonomic responses (as well as on the symptomatic responses) to hypoglycemia. Both studies indicate that the adrenomedullary (epinephrine) response to hypoglycemia is reduced to some extent after exercise on the previous day. Although the present data do not indicate that the sympathetic neural (norepinephrine) response is reduced, Galassetti et al. (23) found the muscle sympathetic nerve activity response (as well as the plasma norepinephrine response) to be reduced. The microneurographic measurement of muscle sympathetic nerve activity is probably a more sensitive measure of sympathetic neural activation than the plasma norepinephrine concentration (37).

Thus, the data are qualitatively consistent with the hypothesis of Davis et al. (20,21) that cortisol at least in part mediates the reduced autonomic component of HAAF (10–14). However, the limited impact of previous exercise and the resulting transient increase in plasma cortisol on the key glucose counterregulatory hormone responses to subsequent hypoglycemia—no effect on the glucagon response and a small reduction of the epinephrine response in the present data—suggest that an additional factor or factors may well be involved in the pathogenesis of the reduced autonomic component of HAAF.

Although the biological impact of the observed small reduction in the epinephrine response to hypoglycemia after exercise on the previous day is open to question, significantly higher glucose infusion rates were required to maintain the hypoglycemic clamps on the day after exercise. That finding indicates increased responsiveness to insulin on the day after exercise. The extent to which that was the result of the reduced epinephrine response or some other mechanism is unknown.

In contrast to the impact of previous exercise on the

epinephrine response to hypoglycemia, exercise had no effect on the symptomatic responses to hypoglycemia on the next day. Importantly, neurogenic (autonomic) symptom scores—largely the result of the perception of physiologic changes caused by the autonomic (adrenomedullary and sympathetic neural) discharge triggered by hypoglycemia (36)—were no different during hypoglycemia on the day after exercise from those during hypoglycemia on the day after rest. Galassetti et al. (23) also found no effect of previous exercise on the symptomatic responses to hypoglycemia. Thus, it seems that the reduced neurogenic symptom component of HAAF (20–22) is not mediated by cortisol.

In summary, the present data provide some additional support for the hypothesis that the cortisol response to hypoglycemia mediates in part the reduced autonomic response to subsequent hypoglycemia (20–22), one key component of the clinical concept of HAAF in type 1 diabetes (10–16). However, the small effect of vigorous exercise-induced cortisol release on the epinephrine response—with no significant effect on the norepinephrine or pancreatic polypeptide responses—to subsequent hypoglycemia observed suggests that an additional factor or factors may well be involved. Furthermore, the present and previous (23) data do not support the hypothesis that the cortisol response to hypoglycemia mediates the reduced neurogenic symptom response to subsequent hypoglycemia, another key component of the concept of HAAF in type 1 diabetes (10–14).

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REFERENCES

1. Cryer PE: Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes* 43:1378–1389, 1994
2. Cryer PE: *Hypoglycemia. Pathophysiology, Diagnosis and Treatment*. New York, Oxford University Press, 1997, p. 85–125
3. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 329:977–986, 1993
4. Intensive blood-glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. The United Kingdom Prospective Diabetes Study Group. *Lancet* 352:837–853, 1998
5. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 182:171–173, 1973
6. Bolli G, De Feo P, Compagnucci P, Cartechini MG, Angeletti F, Santeusanio F, Brunetti P, Gerich JE: Abnormal glucose counterregulation in insulin dependent diabetes mellitus. Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. *Diabetes* 32:134–141, 1983
7. White NH, Skor D, Cryer PE, Bier DM, Levandoski L, Santiago JV: Identification of type 1 diabetic patients at increased risk for hypoglycemia during intensive therapy. *N Engl J Med* 308:485–491, 1983
8. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Massi-Benedetti M, Santeusanio F, Gerich JE, Brunetti P: A reliable and reproducible test for adequate glucose counterregulation in type 1 diabetes. *Diabetes* 33:732–737, 1984
9. Gold AE, MacLeod KM, Frier BM: Frequency of severe hypoglycemia in patients with type 1 diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
10. Cryer PE: Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM: a vicious cycle. *Diabetes* 41:255–160, 1992
11. Dagogo-Jack SE, Craft S, Cryer PE: Hypoglycemia-associated autonomic failure in insulin dependent diabetes mellitus. *J Clin Invest* 91:819–828, 1993
12. Ovalle F, Fanelli CG, Paramore DS, Hershey T, Craft S, Cryer PE: Brief twice weekly episodes of hypoglycemia reduce detection of clinical hypoglycemia in type 1 diabetes mellitus. *Diabetes* 47:1472–1479, 1998
13. Fanelli CG, Paramore DS, Hershey T, Terkamp C, Ovalle F, Craft S, Cryer PE: Impact of nocturnal hypoglycemia on hypoglycemic cognitive dysfunction in type 1 diabetes mellitus. *Diabetes* 47:1920–1927, 1998
14. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo AD, Modarelli F, Lepore M, Annibale B, Ciofetta M, Torlone E, Bottini P, Porcellati F, Santeusanio F, Brunetti P, Bolli GB: Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most neuroendocrine responses to, symptoms of and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 42:1683–1689, 1993
15. Cranston I, Lomas J, Maran A, Macdonald I, Amiel S: Restoration of hypoglycemia unawareness in patients with long duration insulin-dependent diabetes mellitus. *Lancet* 44:283–287, 1994
16. Dagogo-Jack S, Rattarasarn C, Cryer PE: Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 43:1426–1434, 1994
17. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C: Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci U S A* 91:9352–9356, 1994
18. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin dependent diabetes mellitus. *N Engl J Med* 333:1726–1731, 1995
19. Segel SA, Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Powers WJ, Cryer PE: Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. *Diabetes* 50:1911–1917, 2001
20. Davis SN, Shavers C, Costa F, Mosqueda-Garcia R: Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal humans. *J Clin Invest* 98:680–691, 1996
21. Davis SN, Shavers C, Davis B, Costa F: Prevention of an increase in plasma cortisol during hypoglycemia preserves subsequent counterregulatory responses. *J Clin Invest* 100:429–438, 1997
22. Pampanelli S, Lalli C, Del Sindaco P, Lepore M, Ciofetta M, Fanelli C, Brunetti P, Bolli G: Effect of recent antecedent hypoglycemia and responses of cortisol per se on responses to subsequent hypoglycemia in humans (Abstract). *Diabetes* 46:68A, 1997
23. Galassetti P, Mann S, Tate D, Neill RA, Costa F, Wasserman DH, Davis SN: Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol* 280: E908–E917, 2001
24. Schwartz NS, Clutter WE, Shah SD, Cryer PE: The glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest* 79:777–781, 1987
25. Marker JC, Cryer PE, Clutter WE: Simplified measurement of norepinephrine kinetics: application to studies of aging and exercise training. *Am J Physiol* 267:E387, 1994
26. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH: Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes* 26:22–29, 1977
27. Ensinck J: Immunoassays for glucagon. In *Handbook of Experimental Pharmacology*. Vol. 66. Lefebrve P, Ed. New York, Springer Verlag, 1983, p. 203–221
28. Gingerich RL, Lacy PE, Chance RE, Johnson MG: Regional pancreatic concentration and in-vitro secretion of canine pancreatic polypeptide, insulin, and glucagon. *Diabetes* 27:96–101, 1978
29. Schalch D, Parker M: A sensitive double antibody radioimmunoassay for growth hormone in plasma. *Nature (Lond)* 703:1141–1142, 1964
30. Farmer RW, Pierce CE: Plasma cortisol determination: radioimmunoassay and competitive protein binding compared. *Clin Chem* 20:411–414, 1974
31. Shah SD, Clutter WE, Cryer PE: External and internal standards in the

single-isotope derivative (radioenzymatic) measurement of plasma norepinephrine and epinephrine. *J Lab Clin Med* 106:624–629, 1985
32. Hosaka K, Kikuchi T, Mitsuhida N, Kawaguchi A: A new colorimetric method for the determination of free fatty acids with acyl-CoA synthetase and acyl-CoA oxidase. *J Biochem (Tokyo)* 89:1799–1803, 1981
33. Pinter J, Hayaski J, Watson J: Enzymatic assay of glycerol, dihydroxyacetone and glyceraldehyde. *Arch Biochem Biophys* 121:404–414, 1967
34. Lowry O, Passoneau J, Hasselberger F, Schultz D: Effect of ischemia on known substrates and co-factors of the glycolytic pathway of the brain. *J Biol Chem* 239:18–30, 1964
35. Cahill GF Jr, Herrera MG, Morgan AP, Soeldner JS, Steinke J, Levy PL, Reichard GA Jr, Kipnis DM: Hormone-fuel interrelationships during fasting. *J Clin Invest* 45:1751–1769, 1966
36. Towler DA, Havlin CE, Craft S, Cryer P: Mechanisms of awareness of hypoglycemia: perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes* 42:1791–1798, 1993
37. Paramore DS, Fanelli CG, Shah SD, Cryer PE: Forearm norepinephrine spillover during prolonged standing, hyperinsulinemia and hypoglycemia. *Am J Physiol* 275: E872–E881, 1998.