

Estrogen Blunts Neuroendocrine and Metabolic Responses to Hypoglycemia

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This study tested the hypothesis that estrogen is the mechanism responsible for the sexual dimorphism present in the neuroendocrine and metabolic responses to hypoglycemia. Postmenopausal women receiving (E2; $n = 8$) or not receiving (NO E2; $n = 9$) estrogen replacement were compared with age- and BMI-matched male subjects ($n = 8$) during a single-step 2-h hyperinsulinemic-hypoglycemic clamp. Plasma insulin (599 ± 28 pmol/l) and glucose (2.9 ± 0.03 mmol/l) levels were similar among all groups during the glucose clamp. In response to hypoglycemia, epinephrine (2.8 ± 0.6 vs. 5.8 ± 0.8 and 4.4 ± 0.5 nmol/l), glucagon (57 ± 8 vs. 77 ± 8 and 126 ± 18 ng/l), and endogenous glucose production (2 ± 2 vs. 10 ± 2 and 6 ± 3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were significantly lower in E2 vs. both NO E2 and male subjects ($P < 0.05$). These reduced counterregulatory responses resulted in significantly greater glucose infusion rates (16 ± 2 vs. 6 ± 2 and 6 ± 3 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.01$) in E2 vs. both NO E2 and male subjects. Pancreatic polypeptide was significantly lower ($P < 0.05$) in both the E2 and NO E2 groups compared with the male subjects (136 ± 20 and 136 ± 23 vs. 194 ± 16 pmol/l). Last, glycerol (36 ± 3 vs. 47 ± 5 $\mu\text{mol/l}$; $P < 0.05$), lactate (1.4 ± 0.1 vs. 1.8 ± 0.2 mmol/l; $P < 0.05$), and muscle sympathetic nerve activity (19 ± 4 to 27 ± 4 vs. 27 ± 5 to 42 ± 6 bursts/min; $P < 0.05$) responses to hypoglycemia were all significantly lower in E2 vs. NO E2 subjects. We conclude that estrogen appears to play a major role in the sexual dimorphism present in counterregulatory responses to hypoglycemia in healthy humans. *Diabetes* 52:1749–1755, 2003

Men and women respond differently to an acute bout of hypoglycemia. We have previously shown that healthy and type 1 diabetic women, compared with men, have lower catecholamine, glucagon, cortisol, growth hormone, endogenous glucose production (EGP), and lactate responses, and they have increased glycerol responses to hypoglycemia (1,2). This sexual dimorphism also appears to be present in a wide variety of physiological stresses. For

example, women have been found to have reduced neuroendocrine and increased lipolytic responses to exercise (3–5) and reduced sympathetic nervous system responses to cognitive stress (6).

The physiological mechanism(s) responsible for sexually dimorphic responses to stress in humans remains unknown, although it seems likely that one or more of the reproductive hormones may be responsible. Animal studies suggest that estrogen may play an important role. Estrogen administration has been shown to independently reduce catecholamine levels, either by increasing norepinephrine degradation in the brain (and thereby reducing sympathetic system drive) (7) or by decreasing secretion from the adrenal medulla (8,9). Metabolically, estrogen has been found to increase lipolysis (10), glycogen deposition (11), and glucose uptake during exercise in rats (10). Recent studies in mice even suggest that estrogen, specifically estrone sulfate, may have a direct effect on reducing hepatic glucose production by inhibiting hepatic glucose-6-phosphatase activity (12). In contrast to estrogen, progesterone increases fat synthesis (13) and has been found to have no effect on catecholamine secretion from the adrenal medulla (8). In fact, progesterone antagonizes estrogen's effect on glycogen deposition (11), glucose uptake (14), and the ability to increase lipolytic enzyme activity (10). Thus, it is unlikely that progesterone is responsible for the sexual dimorphism. Although testosterone, like estrogen, can also increase lipolysis (13), little information exists regarding testosterone's direct effects on glucose metabolism. Taken together, work from animal studies support the hypothesis that estrogen can exert, either directly or indirectly, profound effects on neuroendocrine systems and intermediary metabolism. Therefore, the aim of this study was to determine whether estrogen is a major *in vivo* mechanism responsible for the sexual dimorphism present in counterregulatory responses to hypoglycemia found in healthy humans.

RESEARCH DESIGN AND METHODS

Subjects. We studied eight postmenopausal women who were taking estrogen-only replacement (E2 group; age 50 ± 2 years, BMI 25 ± 2 kg/m²), nine postmenopausal women who were not taking any hormone replacement therapy (NO E2 group; age 51 ± 1 years, BMI 25 ± 2 kg/m²), and seven male subjects of similar age (47 ± 2 years) and BMI (28 ± 2 kg/m²). Subjects were nonsmokers and were not taking any medications other than estrogen replacement in the E2 group. All subjects had normal electrocardiogram stress tests responses, normal liver, and normal renal and hematological parameters. The duration of postmenopausal status for the E2 and NO E2 group was 4 ± 2 and 8 ± 5 years, respectively. Duration of estrogen replacement in the E2 group was 4 ± 2 years. Two women in the E2 group had previous total hysterectomies 8 and 10 years before the study. The type of estrogen replacement was Premarin ($n = 3$, conjugated estrogens), Estrace ($n = 3$,

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EGP, endogenous glucose production; HPLC, high-performance liquid chromatography; MSNA, muscle sympathetic nerve activity; NEFA, nonesterified fatty acid; R_a , rate of glucose appearance.

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estradiol), or the Vivid Patch ($n = 2$, estradiol). Studies were approved by the Vanderbilt University human subjects institutional review board, and all subjects gave informed written and verbal consent.

Experimental design. Subjects did not exercise and consumed their usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt University Clinical Research Center the evening before an experiment. The next morning, subjects had one intravenous cannula placed into each hand under local 1% lidocaine anesthesia. One cannula was placed in a retrograde fashion into a vein in the back of the hand. This hand was placed in a heated box (55–60°C) so that arterialized blood could be obtained (15). The other cannula was placed in the contralateral arm for infusions of dextrose, insulin, and labeled glucose during the experiment.

Each study consisted of a tracer equilibration period (0–90 min), a basal period (90–120 min), and an experimental period (120–240 min). A primed (18 μCi) infusion (0.18 $\mu\text{Ci}/\text{min}$) of high-pressure liquid chromatography (HPLC)-purified [^3H]glucose (11.5 $\text{mCi} \cdot \text{mmol}^{-1} \cdot \text{l}^{-1}$; Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) starting at 0 min. Also at this time, isolation of the peroneal nerve for microneurography (technique described below) was started. An insulin infusion solution was prepared with normal saline containing 3% (vol/vol) of the subject's own plasma. At time 120 min, a primed constant (9.0 $\text{pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) infusion of insulin (Eli Lilly, Indianapolis, IN) was started via a precalibrated infusion pump (Harvard Apparatus) and continued until 240 min. The rate of fall of glucose was controlled (0.06 mmol/min) and the hypoglycemic nadir (2.9 mmol/l) achieved using a modification of the glucose clamp technique (16). During the clamp periods, plasma glucose was measured every 5 min, and a 20% dextrose infusion was adjusted so that plasma glucose levels were held constant (2.9 ± 0.1 mmol/l). Potassium chloride (20 mmol/l) was infused during the clamp to reduce insulin-induced hypokalemia.

Tracer calculations. The rate of glucose appearance (R_a), EGP, and glucose utilization were calculated according to the methods of Wall et al. (17). 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 it from 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 eliminates most, if not all, of the problems. In addition, to maintain a constant specific activity, isotope delivery was increased commensurate with increases in exogenous glucose infusion. During this study, only glucose flux results from the basal and the final 30-min periods of the hypoglycemic clamps are reported.

Direct measurement of muscle sympathetic nerve activity. Muscle sympathetic nerve activity (MSNA) was recorded in the present study because this has been demonstrated to reflect increased sympathetic activity during insulin-induced hypoglycemia (2,18–21). MSNA was measured in the peroneal nerve at the level of the fibular head or popliteal fossa. A recording of MSNA was considered adequate when 1) there was spontaneous appearance of pulse-linked bursts, 2) nerve activity increased during phase II (hypotensive phase) and was suppressed during phase IV (blood pressure overshoot) of the Valsalva maneuver, 3) nerve activity increased in response to held expiration (apnea), 4) there was insensitivity to emotional stimuli (loud yell or clap), and/or 5) stretching of the tendons in the foot or tapping the muscle belly evoked proprioceptive afferent signals, whereas cutaneous stimulation by stroking the skin did not.

Sympathetic nerve activity is expressed as bursts per minute. Measurements of MSNA were made from original tracings or on-line recordings (DI-220; Dataq Instruments, Akron, OH) by an operator blinded to the sequence of experiments. Bursts were selected if the signal-to-noise ratio was greater than 2:1.

Analytical methods. The collection and processing of blood samples have been previously described (22). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood for hormones and intermediary metabolites were drawn twice during the control period and every 15 min during the experimental period. Glucagon was measured according to the method of Aguilar-Parada et al. (23), with an interassay coefficient of variation (CV) of 15%. Insulin was measured as previously described (24), with an interassay CV of 11%. Catecholamines were determined by HPLC (25), with an interassay CV of 12% for both epinephrine and norepinephrine. 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 epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Cortisol (Clinical Assays Gamma Coat radioimmuno-

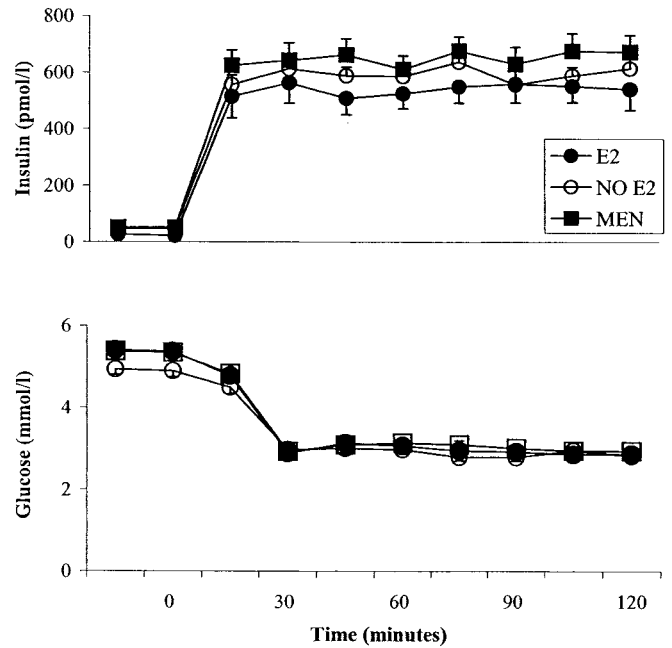


FIG. 1. Glucose and insulin levels during the 2-h hyperinsulinemic ($1.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) hypoglycemic clamp in women taking estrogen (E2), women not taking estrogen (NO E2), and male subjects. Levels were similar during the clamp between all three groups. Values are means \pm SE.

assay kit, interassay CV = 6%), growth hormone (26) (interassay CV = 8%), pancreatic polypeptide (interassay CV = 8%) (27), glucagon (interassay CV = 15%; Linco Research, St. Louis, MO), and leptin (interassay CV = 8%) (28) were all measured using radioimmunoassay techniques. Lactate, glycerol, alanine, and 3-hydroxybutyrate were measured on deproteinized whole blood, using the method of Lloyd et al. (29). Nonesterified fatty acids (NEFAs) were measured using a Wako kit adapted for use on a centrifugal analyzer (30).

Cardiovascular parameters (heart rate and systolic, diastolic, and mean arterial pressure) were measured noninvasively by a Dinamap (Critikon, Tampa, FL) every 10 min throughout each 240-min study. Symptoms of hypoglycemia were assessed every 15 min (31) during the hypoglycemic clamps, using a previously validated semiquantitative questionnaire. Each subject was asked to rate symptoms of tiredness, confusion, hunger, dizziness, difficulty in thinking, blurred vision, sweatiness, tremors, agitation, feeling hot/thirsty, and palpitations. The scores for the first six symptoms were summed for the neuroglycopenic score, and the scores for the last five symptoms were summed for the autonomic symptom score.

Statistical analysis. Data are expressed as means \pm SE and were analyzed using standard parametric one-way ANOVA and with repeated measures where appropriate. A Tukey's post hoc analysis was used delineate statistical significance. A P value ≤ 0.05 was accepted as statistically significant.

RESULTS

Basal E2 levels. Estradiol levels were significantly greater in the E2 group (141 ± 21 pg/ml) compared with both NO E2 (31 ± 7 pg/ml) and male subjects (48 ± 7 pg/ml , $P < 0.05$).

Glucose and insulin levels. Steady-state plasma glucose (2.9 ± 0.1 , 2.9 ± 0.1 , and 2.9 ± 0.1 mmol/l for E2, NO E2, and male subjects, respectively) and insulin (552 ± 65 , 587 ± 30 , and 660 ± 60 pmol/l for E2, NO E2, and male subjects, respectively) levels were similar between the three groups (Fig. 1).

Counterregulatory hormone levels. In response to hypoglycemia, steady-state epinephrine, glucagon (Fig. 2), norepinephrine, growth hormone, cortisol (Table 1), and pancreatic polypeptide (Fig. 3) levels were significantly greater than baseline values in all groups ($P < 0.01$).

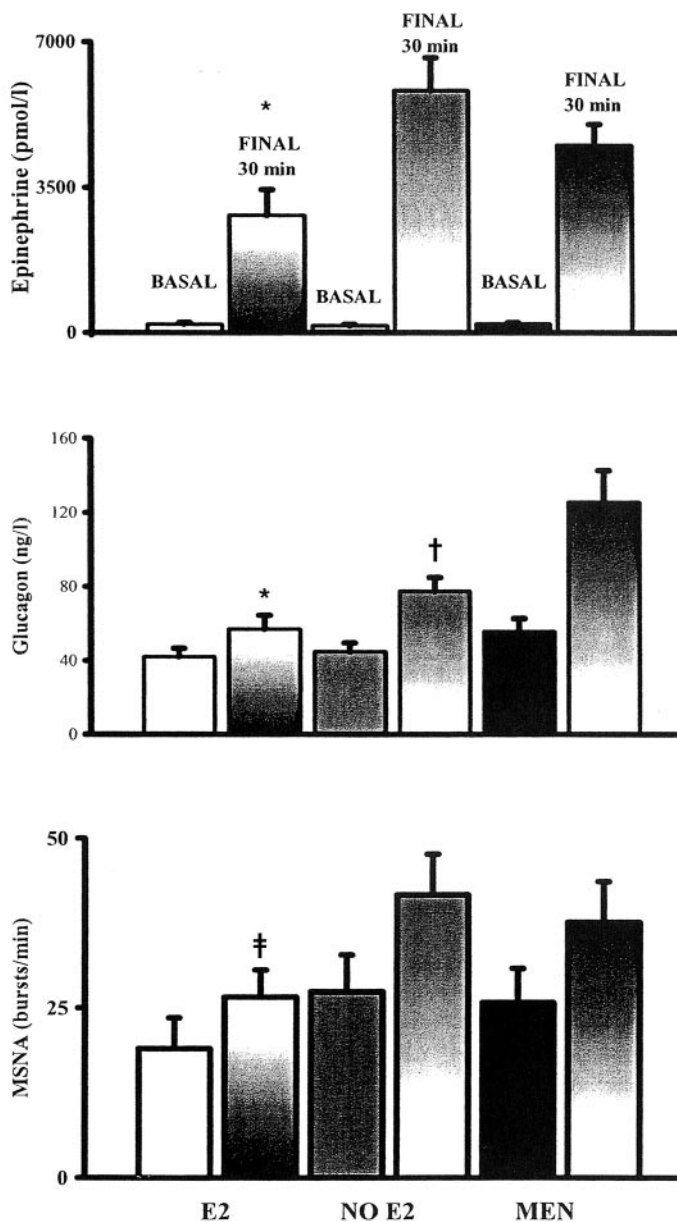


FIG. 2. Epinephrine and glucagon levels and MSNA during the 2-h hyperinsulinemic ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) hypoglycemic clamp in women taking estrogen (E2), women not taking estrogen (NO E2), and male subjects. *Significantly lower in E2 versus both NO E2 and male subjects ($P < 0.01$); †significantly lower in NO E2 versus male subjects; ‡significantly lower in E2 versus NO E2 subjects ($P < 0.05$). Values are means \pm SE.

However, the rise in epinephrine and glucagon (Fig. 2) was significantly lower in E2 vs. both NO E2 and male subjects, and glucagon levels were significantly lower in NO E2 vs. male subjects ($P < 0.01$). Pancreatic polypeptide was significantly lower in both E2 and NO E2 groups vs. the male subjects ($P < 0.05$) (Fig. 3). Leptin levels did not change during hypoglycemia in E2 subjects as compared with the significant reductions seen in NO E2 and male subjects ($P < 0.05$) (Fig. 3).

MSNA. MSNA significantly increased with hypoglycemia in all groups (E2 group 19 ± 4 to 27 ± 4 bursts/min, NO E2 group 27 ± 5 to 42 ± 6 bursts/min, and male subjects 25 ± 5 to 38 ± 6 bursts/min). The increase of MSNA in E2

TABLE 1
Effects of estrogen replacement therapy on norepinephrine, cortisol, and growth hormone responses to hypoglycemia

	E2 subjects	NO E2 subjects	Male subjects
Norepinephrine (nmol/l)			
Basal	1.5 ± 0.3	1.2 ± 0.2	1.4 ± 0.3
Final 30 min	2.3 ± 0.3	2.1 ± 0.2	2.6 ± 0.3
Cortisol (nmol/l)			
Basal	340 ± 30	245 ± 38	260 ± 30
Final 30 min	705 ± 79	774 ± 37	715 ± 37
Growth hormone ($\mu\text{g/ml}$)			
Basal	2.5 ± 1.4	0.2 ± 0.04	0.7 ± 0.3
Final 30 min	12 ± 5	11 ± 5	15 ± 4

Data are means \pm SE.

subjects was significantly reduced ($P < 0.05$) compared with NO E2 subjects (Fig. 2).

Glucose kinetics. Glucose specific activity (disintegrations $\cdot \text{min}^{-1} \cdot \text{mmol}^{-1}$) did not significantly change during both the control period and the final 30 min of the hypoglycemic clamp (CV = 4%) (Table 2). EGP was significantly decreased during hyperinsulinemic hypoglycemia in the E2 vs. both NO E2 and male subjects (Fig. 4). Glucose utilization increased with hypoglycemia but was similar between the three groups. As a consequence of the reduced EGP, the exogenous glucose infusion rate was twice as great in E2 vs. NO E2 or the male subjects (16 ± 2 vs. 7 ± 2 and $7 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.01$) (Fig. 4).

Intermediary metabolism. β -Hydroxybutyrate and NEFA levels both decreased with hyperinsulinemic hypoglycemia ($P < 0.01$) (Table 3). However, despite this decrease, NEFA levels were significantly greater in male subjects versus both E2 and NO E2 subjects. Glycerol and lactate levels increased with hypoglycemia, and this increase was significantly lower in E2 vs. NO E2 subjects ($P < 0.05$) (Table 3).

Cardiovascular responses. Heart rate increased with hypoglycemia in all groups compared with the control period ($P < 0.05$) (Table 4). During the final 20 min of the clamp, systolic blood pressure was significantly lower in E2 vs. male subjects ($P < 0.05$) (Table 4), whereas diastolic blood pressure fell with hypoglycemia in all groups ($P < 0.05$) (Table 4). Mean arterial pressure fell with hypoglycemia in both the E2 and NO E2 groups ($P < 0.05$) (Table 4), but there was no significant change in mean arterial pressure in the male subjects.

Symptom responses. Total symptom scores increased with hypoglycemia from 15 ± 1 to 28 ± 4 in E2 subjects, from 15 ± 1 to 24 ± 2 in NO E2 subjects, and from 19 ± 2 to 32 ± 5 in male subjects. Neurogenic and neuroglycopenic symptom scores contributed evenly to the total symptom score and were not different between the three groups.

DISCUSSION

This study examined the role of estrogen in the sexual dimorphism present in counterregulatory responses to hypoglycemia in healthy humans. The main findings were that postmenopausal women taking estrogen replacement had reduced epinephrine, glucagon, MSNA, pancreatic

TABLE 2
Specific activity during the basal and final 30-min periods of the hyperinsulinemic-hypoglycemic clamps

Specific activity (dpm/mmol)	-30	-20	-10	0	90	105	120
E2 subjects	614 ± 54	627 ± 58	604 ± 50	604 ± 50	381 ± 34	351 ± 33	351 ± 35
NO E2 subjects	536 ± 26	530 ± 22	537 ± 23	502 ± 26	363 ± 31	364 ± 31	358 ± 31
Male subjects	423 ± 61	418 ± 63	413 ± 52	402 ± 58	294 ± 26	301 ± 28	301 ± 25

Data are means ± SE.

polypeptide, leptin, EGP, NEFA, lactate, glycerol, and mean arterial blood pressure responses to hypoglycemia compared with age- and weight-matched postmenopausal women not taking estrogen replacement and/or compared with male subjects. Thus, estrogen appears to be a major in vivo mechanism responsible for the reduced neuroendocrine and metabolic responses to hypoglycemia occurring in healthy women as compared with men.

The pattern of sexually dimorphic results observed in the present study is similar to previous studies in premenopausal women. We have observed reduced epinephrine, MSNA, pancreatic polypeptide, glucagon, EGP, and lactate responses to hypoglycemia (1,2,32) in premenopausal women compared with age-matched men. Other laboratories have also shown reduced epinephrine (33–35) and glucagon (35) responses to hypoglycemia in women compared with men. In the current study, the E2 group had significantly reduced epinephrine, glucagon, and EGP compared with both NO E2 and male subjects. However, interestingly, there were also differences between NO E2 and male subjects (glucagon and pancreatic polypeptide).

Thus, for the majority of counterregulatory variables, estrogen plays an important role in sexually dimorphic responses to hypoglycemia, but the contribution of other factors to regulating differences in glucagon and pancreatic polypeptide cannot be ruled out.

Estrogen's regulation of epinephrine and MSNA responses to hypoglycemia may be of central (i.e., brain) and/or peripheral origin. This is illustrated by in vitro data showing estrogen administration reduced catecholamine secretion directly from the adrenal gland (9). Other studies have also demonstrated reduced norepinephrine release from the hypothalamus after estrogen administration (7). Estrogen may also act indirectly by altering brain glucose transport. Rats given estrogen and then exposed to ischemic injury have been found to have increased GLUT1 receptors in the brain (36), thereby increasing brain glucose transport. If estrogen could increase glucose transport within the brain during hypoglycemia, the stimulus to activate the sympathetic drive would be reduced, and thus the resulting sympathetic counterregulatory responses would be lowered.

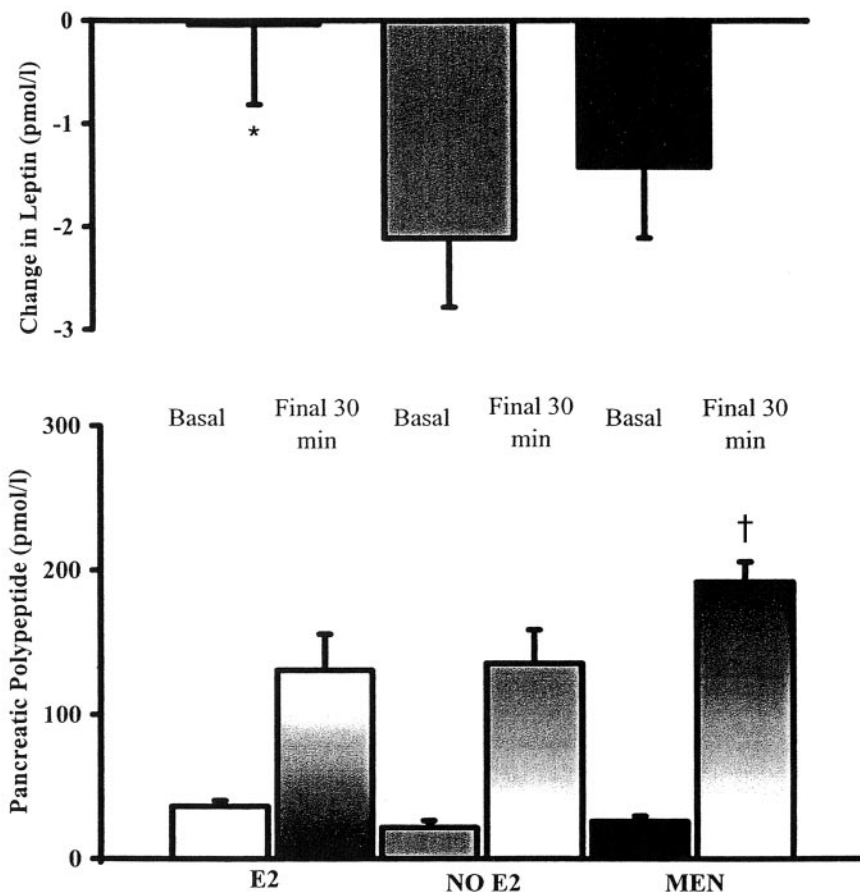


FIG. 3. The change in leptin levels and absolute pancreatic polypeptide levels during the 2-h hyperinsulinemic ($9 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) hypoglycemic clamp in women taking estrogen (E2), women not taking estrogen (NO E2), and male subjects. *The change in leptin was significantly less in E2 versus both NO E2 and male subjects ($P < 0.01$). †Pancreatic polypeptide levels were significantly lower in E2 and NO E2 versus male subjects ($P < 0.01$). Values are means ± SE.

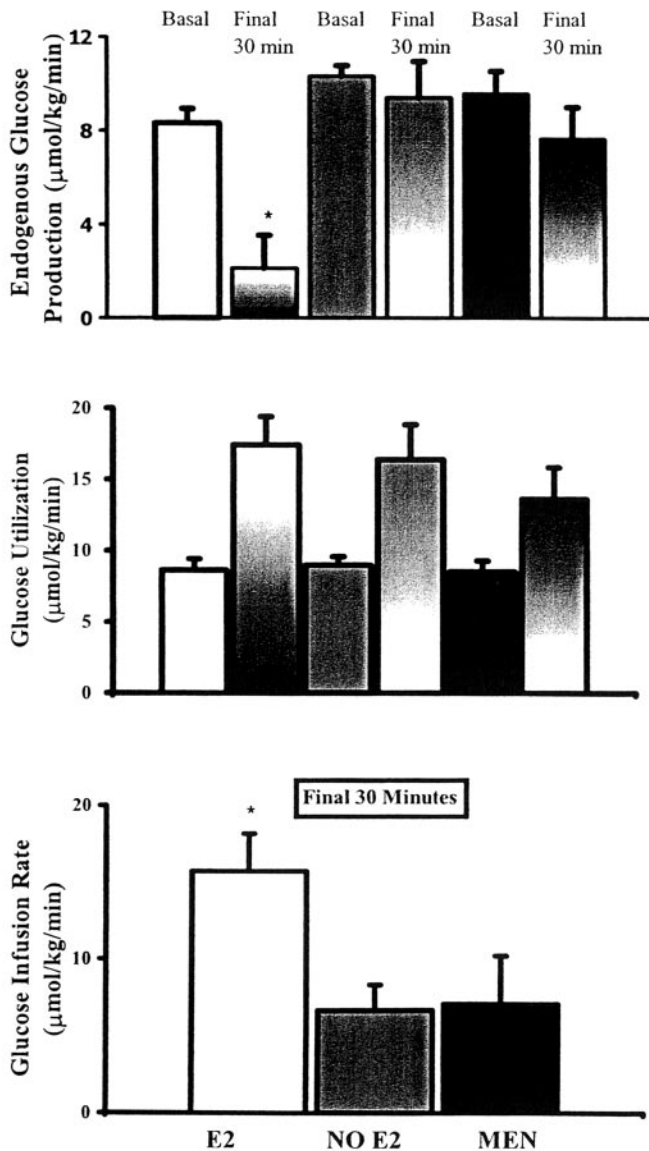


FIG. 4. EGP, glucose utilization, and glucose infusion rate during the 2-h hyperinsulinemic ($9 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) hypoglycemic clamp in women taking estrogen (E2), women not taking estrogen (NO E2), and male subjects. *The EGP and glucose infusion rates were significantly lower and higher, respectively, in E2 versus both NO E2 and male subjects ($P < 0.01$). Values are means \pm SE.

Besides the impact on the sympathetic nervous system, other endocrine responses were also altered in the E2 group. First, the fall in leptin levels was significantly less in E2 subjects compared with both NO E2 and male subjects. In vitro studies suggest that estradiol administration can increase leptin expression in adipose tissue (37). In contrast, epinephrine has been shown to reduce leptin levels (38). Thus, the presence of estrogen plus the lack of epinephrine may have prevented a fall in leptin levels during hypoglycemia in the E2 group. Second, although there is a possibility that estrogen could directly inhibit glucagon secretion during hypoglycemia, it is also possible that the lack of epinephrine contributed to the reduced glucagon levels in the E2 group. Finally, estrogen has been suggested to increase baseline growth hormone levels (39), and we did see this trend between E2 and NO E2 subjects in baseline growth hormone levels when the men

TABLE 3
Effects of estrogen replacement therapy on metabolite responses to hypoglycemia

	E2 subjects	NO E2 subjects	Male subjects
Alanine ($\mu\text{mol/l}$)			
Basal	278 ± 26	269 ± 17	307 ± 35
Final 30 min	268 ± 15	286 ± 18	295 ± 20
β -Hydroxybutyrate ($\mu\text{mol/l}$)			
Basal	175 ± 59	159 ± 41	75 ± 19
Final 30 min	10 ± 2	13 ± 2	13 ± 2
Glycerol (mmol/l)			
Basal	53 ± 4	60 ± 6	49 ± 7
Final 30 min	$36 \pm 3^*$	48 ± 4	43 ± 6
Lactate (mmol)			
Basal	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Final 30 min	$1.4 \pm 0.1^*$	1.7 ± 0.2	1.6 ± 0.1
Nonesterified free fatty acids ($\mu\text{mol/l}$)			
Basal	574 ± 71	535 ± 32	558 ± 57
Final 30 min	153 ± 47	117 ± 14	$244 \pm 40^\ddagger$

Data are means \pm SE. *Significantly lower in E2 versus NO E2 subjects ($P < 0.05$); ‡ significantly greater than E2 and NO E2 subjects.

were removed from the analysis ($P = 0.07$). However, we have previously reported that women have a decreased, rather than an increased, growth hormone response to hypoglycemia (2). Because aging decreases growth hormone levels (39), the growth hormone responses to hypoglycemia in this study were significantly truncated compared with our earlier work. This reduced the experimental signal and possibly prevented the detection of any differences in response to hypoglycemia among the groups.

Because epinephrine is a key counterregulatory hormone, estrogen-induced changes in epinephrine could have major consequences for glucose and fat metabolism in response to hypoglycemia. The lack of a rise in epineph-

TABLE 4
Effects of estrogen on cardiovascular responses to hypoglycemia

	Basal period	Duration of hypoglycemia (min)		
		80	100	120
Heart rate (bpm)				
E2	67 ± 3	$75 \pm 4^*$	$71 \pm 5^*$	$73 \pm 5^*$
NO E2	66 ± 4	$71 \pm 4^*$	$70 \pm 3^*$	$74 \pm 6^*$
Men	62 ± 3	$72 \pm 4^*$	$70 \pm 4^*$	$74 \pm 4^*$
Systolic BP (mmHg)				
E2	116 ± 6	105 ± 7	107 ± 7	106 ± 8
NO E2	124 ± 8	113 ± 6	112 ± 7	112 ± 7
Men	120 ± 6	$128 \pm 7^\ddagger$	$123 \pm 6^\ddagger$	$124 \pm 8^\ddagger$
Diastolic BP (mmHg)				
E2	69 ± 4	$58 \pm 4^\ddagger$	$61 \pm 3^\ddagger$	$60 \pm 4^\ddagger$
NO E2	73 ± 4	$60 \pm 4^\ddagger$	$57 \pm 3^\ddagger$	$57 \pm 3^\ddagger$
Men	70 ± 3	$62 \pm 3^\ddagger$	$62 \pm 3^\ddagger$	$60 \pm 4^\ddagger$
Mean arterial BP (mmHg)				
E2	84 ± 5	$74 \pm 6^\ddagger$	$75 \pm 3^\ddagger$	$75 \pm 4^\ddagger$
NO E2	90 ± 6	$77 \pm 5^\ddagger$	$75 \pm 4^\ddagger$	$76 \pm 4^\ddagger$
Men	87 ± 4	84 ± 4	82 ± 4	81 ± 5

Data are means \pm SE. *Significantly greater than basal period ($P < 0.05$); ‡ significantly greater than E2 ($P < 0.05$); ‡ significantly lower than basal period ($P < 0.05$). BP, blood pressure.

rine could explain the reduced EGP and lactate responses in E2 vs. NO E2 and male subjects. However, glucagon is also an important regulator of EGP. Thus, the lack of a rise in both of these hormones with hypoglycemia most likely contributed to the substantial reduction in EGP. Additionally, a direct effect of estrogen cannot be ruled out because the hormone has been shown to reduce hepatic glucose production by decreasing glucose-6-phosphatase and thus gluconeogenesis (12).

Regarding fat metabolism, epinephrine is also a major lipolytic activator during hypoglycemia. Glycerol levels, an index of whole-body lipolysis, were reduced in E2 vs. NO E2 subjects. In contrast, NEFA levels were reduced in both E2 and NO E2 subjects compared with male subjects. NEFA levels also rise with lipolysis, but unlike glycerol levels, they are subject to insulin-induced reesterification. Although estrogen has been shown to increase basal lipolysis in rats (10), the fact that glycerol and NEFA levels were reduced in the E2 group suggest that the blunted epinephrine and sympathetic nervous system responses to hypoglycemia overrode any stimulatory effect of estrogen on lipolysis. Whole-body lipolysis has been shown to be similar between men and women after epinephrine infusion (40,41). Thus, it appears to be that differences in the level of epinephrine, rather than a sexual dimorphism in tissue sensitivity to the hormone, caused the increased lipolysis in the male subjects.

Although estrogen's impact on epinephrine and, consequently, metabolism appears straightforward, its overall influence on the autonomic nervous system as a whole is not so clear. This may be because estrogen is just one of many factors that may influence the autonomic response to hypoglycemia. For example, if estrogen replacement caused a generalized blunting or blunted the sympathetic nervous system, one would expect the E2 group to have lower catecholamines, MSNA, heart rate, blood pressure, and neurogenic (autonomic) symptoms vs. NO E2 and male subjects. Although the E2 group did, in fact, have reduced epinephrine versus both NO E2 and male subjects, lower MSNA versus NO E2 subjects, and lower systolic blood pressure versus the male subjects, we also observed similar norepinephrine, heart rate, and neurogenic symptom responses to hypoglycemia between the three groups. There are many factors that could lead to these results. First, changes in norepinephrine are subject to changes in spillover from the sympathetic nervous system and clearance by the periphery. Differential impacts of sex and/or estrogen on either spillover or clearance could create difficulty in detecting differences in plasma levels between groups. Second, pancreatic polypeptide, a partial marker for the parasympathetic drive, was increased in male subjects in response to hypoglycemia. Regulation of heart rate and blood pressure are controlled through a balance between sympathetic and parasympathetic drives. Therefore, increased parasympathetic drive could offset some of the increased sympathetic drive, leading to similar cardiovascular responses to hypoglycemia. Regulation of blood pressure responses is complex and multifactorial. Systolic blood pressure was lower in the E2 group versus the male subjects, and mean arterial pressure responses were lower in both groups of women compared with the male subjects. The baroreflex is reset

with hypoglycemia (42), and although unknown, it is possible that sex further impacts the normal baroreflex response to hypoglycemia. Alternatively, men have been found to have greater responsiveness to epinephrine-induced changes in blood pressure (41) and norepinephrine-induced vasoconstriction (43), suggesting that men may simply be more sensitive to catecholamine-induced changes in blood pressure. We have previously reported dissociation between plasma catecholamines, MSNA, and neurogenic symptom responses to hypoglycemia (18). Therefore, the finding that epinephrine and MSNA but not neurogenic symptom responses were reduced during hypoglycemia is consistent with our previous data. Thus, although the E2 group had some reduced markers of both sympathetic (i.e., epinephrine and MSNA) and parasympathetic (i.e., pancreatic polypeptide) branches of the autonomic nervous system, other more complexly controlled functions (i.e., norepinephrine, cardiovascular, and symptom responses) were similar among the groups.

It is interesting to note that despite the older age group in this versus our previous studies (1,2,32), we saw similar sex differences. It is also important to note that these groups had similar BMI levels. This suggests that body fat and age, per se, do not appear to be major mechanisms responsible for the sex differences seen in response to hypoglycemia.

In summary, the present results show that postmenopausal women taking estrogen replacement have reduced counterregulatory responses to hypoglycemia compared with both women not taking estrogen replacement and men. Women taking estrogen replacement had reduced epinephrine, MSNA, pancreatic polypeptide, glucagon, EGP, lactate, and glycerol responses to hypoglycemia compared with women not taking estrogen replacement and/or compared with men. In conclusion, these results support the hypothesis that estrogen is a major mechanism responsible for the sexual dimorphism present in neuroendocrine and metabolic counterregulatory responses to hypoglycemia in healthy humans.

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REFERENCES

1. Davis SN, Fowler S, Costa F: Hypoglycemic counterregulatory responses differ between men and women with type 1 diabetes. *Diabetes* 49:65-72, 2000
2. Davis SN, Shavers C, Costa F: Differential gender responses to hypoglycemia are due to alterations in CNS drive and not glycemic thresholds. *Am J Physiol Endocrinol Metab* 279:E1054-E1063, 2000
3. Davis SN, Galassetti P, Wasserman DH, Tate D: Effect of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. *J Clin Endocrinol Metab* 85:224-230, 2000
4. Horton TJ, Pagliassotti MJ, Hobbs K, Hill JO: Fuel metabolism in men and

- women during and after long-duration exercise. *J Appl Physiol* 85:1823–1832, 1998
5. Tarnopolsky L, MacDougall S, Atkinson S, Tarnopolski M, Sutton J: Gender differences in substrate for endurance exercise. *J Appl Physiol* 68:302–308, 1990
 6. Fagius J, Berne C: The increase in sympathetic nerve activity after glucose ingestion is reduced in type 1 diabetes. *Clin Sci* 98:627–632, 2000
 7. Vathy I, Etgen AM: Ovarian steroids and hypothalamic norepinephrine release: studies using in vivo brain microdialysis. *Life Sci* 43:1493–1499, 1988
 8. Dar DE, Zinder O: Short term effect of steroids on catecholamine secretion from bovine adrenal medulla chromaffin cells. *Neuropharmacology* 36:1783–1788, 1997
 9. de Miguel R, Fernandez-Ruiz JJ, Hernandez ML, Ramos JA: Role of ovarian steroids on the catecholamine synthesis and release in female rat adrenal: in vivo and in vitro studies. *Life Sci* 44:1979–1986, 1989
 10. Campbell SE, Febbraio M: Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol Endocrinol Metab* 281:E803–E808, 2001
 11. Carrington LJ, Bailey CJ: Effects of natural and synthetic estrogens and progestins on glycogen deposition in female mice. *Hormone Res* 21:199–203, 1985
 12. Borthwick EB, Houston MP, Coughtrie MW, Burchell A: The antihyperglycemic effect of estrone sulfate in genetically obese-diabetic (ob/ob) mice is associated with reduced hepatic glucose-6-phosphatase. *Horm Metab Res* 33:721–726, 2001
 13. Hansen FM, Fahmy N, Nielsen JH: The influence of sexual hormones on lipogenesis and lipolysis in rat fat cells. *Acta Endocrinol (Copenh)* 95:566–570, 1980
 14. Campbell SE, Febbraio MA: Effect of ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake. *Am J Physiol Endocrinol Metab* 282:E1139–E1146, 2001
 15. Abumrad NN, Rabin D, Diamond MC, Lacy WW: Use of a heated superficial hand vein as an alternative site for measurement of amino acid concentration and for the study of glucose and alanine kinetics in man. *Metabolism* 30:936–940, 1981
 16. DeFronzo RA, Tobin K, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E216–E223, 1979
 17. Wall JS, Steele R, DeBodo RD, Altszuler N: Effect of insulin on utilization and production of circulating glucose. *Am J Physiol* 189:43–50, 1957
 18. Davis SN, Mann S, Galassetti P, Neill RA, Tate D, Ertl AC, Costa F: Effects of differing durations of antecedent hypoglycemia on counterregulatory responses to subsequent hypoglycemia in normal humans. *Diabetes* 49:1897–1903, 2000
 19. Davis SN, Shavers C, Mosqueda-Garcia R, Costa F: Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. *Diabetes* 46:1328–1335, 1997
 20. Galassetti P, Mann S, Tate D, Neill RA, Costa F, Wasserman DH, Davis SN: Effect of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. *Am J Physiol* 280:E908–E917, 2001
 21. Galassetti P, Neill RA, Tate D, Ertl AC, Wasserman DH, Davis SN: Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *J Clin Endocrinol Metab* 86:3516–3524, 2001
 22. Cherrington AD, Williams PE, Harris MS: Relationship between the plasma glucose level and glucose uptake in the conscious dog. *Metabolism* 27:787–791, 1978
 23. Aguilar-Parada E, Eisentraut AM, Unger RH: Pancreatic glucagon secretion in normal and diabetic subjects. *Am J Med Sci* 257:415–419, 1969
 24. Wide L, Porath J: Radioimmunoassay of proteins with the use of sephadex-coupled antibodies. *Biochim Biophys Acta* 130:257–260, 1966
 25. Causon R, Caruthers M, Rodnight R: Assay of plasma catecholamines by liquid chromatography with electrical detection. *Anal Biochem* 116:223–226, 1982
 26. Hunter W, Greenwood F: Preparation of [¹³¹I]-labeled human growth hormone of high specific activity. *Nature* 194:495–496, 1962
 27. Hagopian W, Lever E, Cen D, Emmounoud D, Polonsky K, Pugh W, Moosa A, Jaspan JB: Predominance of renal and absence of hepatic metabolism of pancreatic polypeptide in the dog. *Am J Physiol* 245:171–177, 1983
 28. Davis SN, Geho B, Tate D, Galassetti P, Lau J, Grammer D, Mann S: The effects of HDV-insulin on carbohydrate metabolism in type 1 diabetic patients. *J Diabetes Complications* 15:227–233, 2001
 29. Lloyd B, Burrin J, Smythe P, Alberti KGM: Enzymatic fluorometric continuous-flow assays for blood glucose lactate, pyruvate, alanine, glycerol, and 3-hydroxybutyrate. *Clin Chem* 24:1724–1729, 1978
 30. Ho RJ: Radiochemical assay of long chain fatty acid using ⁶³Ni as tracer. *Anal Biochem* 26:105–113, 1970
 31. Cox DJ, Gonder-Frederick L, Antoun B, Cryer PE, Clarke WL: Perceived symptoms in the recognition of hypoglycemia. *Diabetes Care* 16:519–527, 1993
 32. Davis SN, Shavers C, Costa F: Gender-related differences in counterregulatory responses to antecedent hypoglycemia in normal humans. *J Clin Endocrinol Metab* 85:2148–2157, 2000
 33. Amiel SA, Maran A, Powrie JK, Umpleby AM, Macdonald IA: Gender differences in counterregulation to hypoglycemia. *Diabetologia* 36:460–464, 1993
 34. Diamond MP, Jones T, Caprio S, Hallarman L, Diamond MC, Addabbo M, Tamborlane WV, Sherwin RS: Gender influences counterregulatory hormone responses to hypoglycemia. *Metabolism* 42:1568–1572, 1993
 35. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, Di Vincenzo A, Annibale B, Lepore M, Lalli C: Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female humans. *Diabetologia* 37:797–807, 1994
 36. Shi J, Zhang YQ, Simpkins JW: Effects of 17 β -estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats. *Exp Brain Res* 117:200–206, 1997
 37. Machinal-Quelin F, Dieudonne MN, Pecquery R, Leneuve MC, Giudicelli Y: Direct in vitro effects of androgens and estrogens on ob gene expression and leptin secretion in human adipose tissue. *Endocrine* 18:179–184, 2002
 38. Fritsche A, Stumvoll M, Grub M, Sieslack S, Renn W, Schmulling RM, Haring HU, Gerich JE: Effect of hypoglycemia on beta-adrenergic sensitivity in normal and type 1 diabetic subjects. *Diabetes Care* 21:1505–1510, 1998
 39. Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO: Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *J Clin Endocrinol Metab* 64:51–58, 1987
 40. Jensen MD, Cryer PE, Johnson CM, Murray MJ: Effects of epinephrine on regional free fatty acid and energy metabolism in men and women. *Am J Physiol* 270:E259–E264, 1996
 41. Webber J, Macdonald IA: A comparison of the cardiovascular and metabolic effects of incremental versus continuous dose adrenaline infusions in men and women. *Int J Obes Relat Metab Disord* 17:37–43, 1993
 42. Fagius J, Berne C: Rapid resetting of human baroreflex working range: insights from sympathetic recordings during acute hypoglycaemia. *J Physiol* 442:91–101, 1991
 43. Kneale BJ, Chowienzyk PJ, Brett SE, Coltart DJ, Ritter JM: Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature. *J Am Coll Cardiol* 36:1233–1238, 2000