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 (595 ± 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 mmol/l), glucagon (57 ± 8 vs. 77 ± 8 and 126 ± 18 ng/l), and endogenous glucose production (2 ± 2 vs. 10 ± 2 and 6 ± 3 μmol·kg⁻¹·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 μmol·kg⁻¹·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 μmol/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), glycerogen 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 ± 5 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, conjugated estrogens).
estriol), or the Vivith Patch (n = 2, estriol). 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 of high-pressure liquid chromatography (HPLC)-purified [3-3H]glucose (11.5 μCi·mmol⁻¹·min⁻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 mmol·kg⁻¹·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 (Rg), EGP, and glucose utilization were calculated according to the methods of Wall et al. (17). EGP was calculated by determining the total Rg (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 Rg and rate of glucose disposal (Rd) 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, isotopic delivery was increased commensurate with increases in exogenous glucose infusion. During this study, only glucose specific 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-related 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 (Digitrac 20; Datag 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 was drawn twice during the control period and every 15 min during the experimental period. Glucagon was measured according to 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 considered for the hypoglycemic score, and the scores for the last five symptoms were summed for the autonomic symptom score.

**Statistical analysis.** Data are expressed as means ± 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 600 ± 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. 1. Glucose and insulin levels during the 2-h hyperinsulinemic (1.5 mU·kg⁻¹·min⁻¹) hypoglycemic clamp in women taking estradiol (E2), women not taking estrogen (NO E2), and male subjects. Levels were similar during the clamp between all three groups. Values are means ± SE.](https://example.com/fig1.png)
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 subjects was significantly reduced (P < 0.05) compared with NO E2 subjects (Fig. 2).

**Glucose kinetics**. Glucose specific activity (disintegrations·min⁻¹·nmol⁻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 ± 3 μmol·kg⁻¹·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

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**TABLE 1**

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

<table>
<thead>
<tr>
<th></th>
<th>E2 subjects</th>
<th>NO E2 subjects</th>
<th>Male subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.5 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Final 30 min</td>
<td>2.3 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>340 ± 30</td>
<td>245 ± 38</td>
<td>260 ± 30</td>
</tr>
<tr>
<td>Final 30 min</td>
<td>705 ± 79</td>
<td>774 ± 37</td>
<td>715 ± 37</td>
</tr>
<tr>
<td>Growth hormone (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.5 ± 1.4</td>
<td>0.2 ± 0.04</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Final 30 min</td>
<td>12 ± 5</td>
<td>11 ± 5</td>
<td>15 ± 4</td>
</tr>
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</table>

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.

### Table 2

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

Data are means ± 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 greater in E2 subjects compared with both 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 ± SE.

Data are means ± 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 epinephrine...
ESTROGEN AND HYPOGLYCEMIA

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, it’s 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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