Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 331–338

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Hormonal, subjective, and neurocognitive responses to brief hypoglycemia in postmenopausal women and age-matched men with type 2 diabetes mellitus

Jan Phillip Bremer[a], Maria Baron[a], Horst Peters[a], Kerstin M. Oltmanns[b], Werner Kern[a], Horst L. Fehm[a], Jan Born[b], Bernd Schultes[a,*]

[a]*Department of Internal Medicine I, University of Luebeck, D-23538 Luebeck, Germany*
[b]*Department of Neuroendocrinology, University of Luebeck, D-23538 Luebeck, Germany*

Received 15 March 2005; accepted 22 September 2005

Abstract

Sexual dimorphisms in hypoglycemic counterregulation are well documented in young healthy and type 1 diabetic subjects. Here, we questioned whether sex differences in counterregulation are present also in type 2 diabetic patients who are in a postmenopausal state. In an attempt to answer this question, we examined hormonal responses to a single-step hypoglycemic clamp (50 mg/dL) in 15 postmenopausal women and 15 age-matched men. Patients were also matched for body mass index, HbA_{1c}, diabetes duration, and diabetes therapy. In addition to hormonal counterregulation, perception of symptoms as well as aspects of neurocognitive function (short-term memory of words and reaction time on an auditory vigilance task) was assessed at baseline and during the hypoglycemic clamp. Hypoglycemia induced a profound rise in almost all counterregulatory hormones, that is, epinephrine, norepinephrine, corticotropin, cortisol, and growth hormone (all $P < .007$), except for glucagon, which slightly decreased ($P = .014$). However, none of the responses differed between sexes (all $P > .256$). In addition, perceived symptoms ($P < .001$) as well as reaction time on the vigilance task ($P < .001$) increased, and short-term memory performance tended to deteriorate ($P = .091$) during hypoglycemia. Again these changes did not differ between the sexes (all $P > .370$). In sum, data suggest that, in contrast to previous observations in young, healthy, and type 1 diabetic subjects, sex does not represent an important determinant of hormonal, subjective, and neurocognitive responses to hypoglycemia in postmenopausal type 2 diabetic patients. However, the women in our study were all postmenopausal and not receiving hormone replacement therapy. Therefore, our results cannot be generalized to female patients with type 2 diabetes who are premenopausal or on hormone replacement therapy, that is, conditions characterized by increased blood estrogen levels.

1. Introduction

Severe hypoglycemic episodes are commonly believed to occur distinctly less frequent in type 2 than in type 1 diabetic patients [1,2]. However, it has been predicted that with increasing efforts to improve glycemic control in patients with type 2 diabetes mellitus (T2DM), the number of severe hypoglycemic episodes will markedly rise in those patients [3,4]. On this background, it appears reasonable to evaluate more closely the factors determining hormonal counterregulation against and awareness of hypoglycemia in patients with T2DM because they influence the risk of severe hypoglycemic episodes in those patients [5,6]. The most comprehensive study on this issue [7] showed that in patients with T2DM, an insulin treatment of longer than 5 years is associated with an attenuated glucagon response to hypoglycemia. Furthermore, results of this study indicated that, similar to observations in patients with type 1 diabetes mellitus (T1DM) [8], in patients with T2DM, glycemic thresholds for counterregulatory activation are also shifted toward lower glucose levels after antecedent hypoglycemic episodes. Another study [9] showed that the glycemic thresholds for hormonal counterregulation and perception of symptoms in patients with T2DM essentially depend on the quality of glycemic control with improved

* Corresponding author. Tel.: +49 451 500 3639; fax: +49 451 500 3640.
E-mail address: schultes@kfg.uni-luebeck.de (B. Schultes).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.09.006

control being associated with higher thresholds (ie, counterregulation starts at lower glucose levels).

Sex may represent another important factor influencing counterregulation against hypoglycemia. Sex-specific differences in the hormonal responses to hypoglycemia are well documented in young subjects with [10] and without [11-13] T1DM. Specifically, in those studies, women have consistently been found to display a weaker hormonal counterregulation than men. Based on these findings, one may expect women to be particularly prone to the experience of severe hypoglycemic episodes. However, to the best of our knowledge, sex differences in incidence rates of hypoglycemic episodes have not been reported in patients with T2DM so far.

In this context, it is interesting that a recent study [14] performed in healthy postmenopausal women with and without estrogen replacement therapy and in age-matched men indicated that an attenuated hormonal response to hypoglycemia in healthy women results, at least in part, from a blunting influence of estrogens on hormonal counterregulation. In light of these findings, the question arises whether women with T2DM, who are frequently in the postmenopausal state, differ from men in their hormonal counterregulatory responses to hypoglycemia. In an attempt to answer this question, we performed single-step hypoglycemic clamp experiments (blood glucose, 50 mg/dL) in 15 postmenopausal women not receiving a hormone replacement therapy (HRT) and 15 men with T2DM. Hormonal counterregulation, symptoms, and aspects of neurocognitive function were assessed at a baseline period and during the standardized hypoglycemia.

2. Methods

2.1. Subjects

We examined 15 postmenopausal women and 15 men matched for age, body mass index, HbA_{1c}, diabetes duration, and diabetes therapy. The patients were recruited by newspaper advertisement and were then screened by physical and routine laboratory examination a few days before the experiments. In women, the postmenopausal state was assumed when the patient reported to not have had a menstrual cycle for at least 1 year and showed a typical combination of high gonadotropin (follicle-stimulating hormone [FSH] >23 mU/mL; luteinizing hormone [LH] >14 mU/mL) and low estrogen (<28 pg/mL) levels in her blood sample. Exclusion criteria for participation in the study were clinical evidence of diabetic neuropathy, overt nephropathy (ie, none of the patients had a macroproteinuria as defined by the ratio of albumin to creatinine in a morning urine sample of >300 mg/g or an elevated serum creatinine level), congestive heart disease, a history of myocardial infarction or stroke, and acute illness of any kind. The clinical characteristics of the patients studied are listed in Table 1.

Table 1
Clinical characteristics of the study population

Variable	Women (n = 15)	Men (n = 15)
Age (y)	61.0 ± 1.9	61.7 ± 2.8
Diabetes mellitus duration (y)	9.1 ± 1.3	9.0 ± 2.3
HbA_{1c} (%)	7.7 ± 0.2	7.6 ± 0.2
Body mass index (kg/m^2)	30.6 ± 1.5	28.6 ± 1.2
Diabetes mellitus therapy		
Diet alone	2	2
Metformin	10	9
Sulfonylurea	4	3
Insulin	7	7
Insulin dose (U/kg per day)	0.44 ± 0.11	0.42 ± 0.08

Data are provided as mean ± SEM and prevalence. Comparison of all variables between women and men did not reach significance (all $P > .15$ by χ^2 or Student t test).

None of the patients declared to have had a severe hypoglycemic episode, defined as a state that required help from another person, during the last year before the experiments. Besides diabetes therapy, other medications taken by the patients were β-blocker (women/men, 6/6), angiotensin-converting enzyme inhibitors (7/5), calcium antagonists (3/3), diuretics (1/4), aspirin and equivalents (4/4), and antilipemic medications (statin and fibrate, 3/3). On the day before the experiment, patients were instructed to ingest their last meal before 7:00 PM. Thereafter, only drinking of water was allowed. In addition, the patients were instructed not to take their medications, except for aspirin or equivalents in the morning of the experiment. All patients gave written informed consent, and the study was approved by the local ethics committee.

2.2. Hypoglycemic clamp procedure

On the day of the experiment, patients reported to the medical research unit at 7:30 AM. The experiment took place in a sound-attenuated room with the patient sitting with the trunk in an almost upright position (~60°) and the legs in a horizontal position on a bed. A cannula was inserted into a vein on the back of the hand, which was placed in a heated box (50°C-55°C) to obtain arterialized venous blood. A second cannula was inserted into an antecubital vein of the contralateral arm. Both cannulae were connected to long thin tubes that enabled blood sampling and adjustment of the rate of dextrose infusion from an adjacent room without being noticed by the subject. After a 30-minute baseline period starting at 8:00 AM, a bolus of 0.08 U human insulin (Insuman Rapid, Aventis, Strasbourg, France) per kilogram of body weight was given over 4 minutes. Thereafter, insulin was infused at a constant rate of 2.5 mU/kg body weight per minute. Blood glucose was allowed to fall to a level of 50 mg/dL and was then maintained at this level for the next 30 minutes. Thus, the time of the hypoglycemic plateau was standardized to a 30-minute period. During this clamp procedure, blood glucose levels were measured every 5 minutes, and a variable infusion of 20% dextrose solution was adjusted to maintain blood glucose at the target level. Immediately after

the 30-minute hypoglycemic plateau, the insulin infusion was stopped and blood glucose levels were normalized to normal levels by increasing the rate of dextrose infusion. Blood samples for determination of insulin, C-peptide, epinephrine, norepinephrine, corticotropin, cortisol, growth hormone, and glucagon in plasma or serum, respectively, were drawn once during the baseline period and every 15 minutes during the 30-minute hypoglycemic plateau.

2.3. Analytic methods

Blood glucose concentration was measured using the glucose dehydrogenase method (HemoCue B-Glucose-Analyzer, Ängelholm, Sweden). Serum insulin, C-peptide, cortisol, and growth hormone concentrations were measured by commercial enzyme-linked immunoassays (all Immulite, DPC, Los Angeles, CA) with the following intra- and interassay coefficients of variation (CVs), respectively: insulin, less than 5.2% and less than 6.1%; C-peptide, less than 7.6% and less than 10.5%; cortisol, less than 5.8% and less than 6.3%; growth hormone, less than 5.8% and less than 5.5%. Plasma corticotropin and glucagon concentrations were also measured by immunoassays (corticotropin [Immulite, DPC]: intra-assay CV, <6.1%; interassay CV, <9.4%; glucagon [Adaltis, Montreal, Canada]: intra-assay CV, <8.0%; interassay CV, <8.2%). Plasma epinephrine and norepinephrine were measured by standard high-performance liquid chromatography with electrochemical detection (Chromosystems, Munich, Germany). Intra- and interassay CVs were less than 2.9% and less than 4.2% for epinephrine and less than 2.6% and less than 3.9% for norepinephrine. Serum LH, FSH, and estradiol concentrations were measured by commercial enzyme-linked immunoassays (all Roche Diagnostics, Mannheim, Germany) with the following intra- and interassay CVs, respectively: LH, <1.8% and <5.2%; FSH, <1.8% and <5.3%; estradiol, <5.7% and 6.2%.

2.4. Symptoms

A semiquantitative symptom questionnaire was presented to the patient once during the baseline period and another time at the end of the 30-minute hypoglycemic plateau. Patients rated from 0 (not at all) to 9 (severely) if they experienced the following 11 symptoms: dizziness, tingling, blurred vision, difficulty to concentrate, faintness, anxiety, palpitation, hunger, sweating, irritability, and tremor. Consistent with categories used by previous investigators [15], the first 5 symptoms were considered neuroglycopenic symptoms, whereas the latter 6 were considered autonomic symptoms. The sum of all individual ratings constituted the total symptom score index.

2.5. Neurocognitive function tests

Neurocognitive function tests comprised a short-term memory task and reaction time on an auditory vigilance task, both of which have previously been shown to be sensitive to the effects of hypoglycemia [16]. To minimize learning effects with repeated testing, all patients had practiced both tasks before proper testing. Neurocognitive function tests were performed at baseline and during the last 15 minutes of the 30-minute hypoglycemic plateau, starting with a short-term memory task. This consisted of the oral presentation of a word list containing 15 words (from a CD player) at a rate of 1 word per second. To enable repeated testing, different lists were formed from a pool of words, as previously described [16,17]. The order of lists was balanced systematically across patients. After a subsequent break of 1 minute, the patient was required to recall verbally all words he/she remembered from the preceding list within 1 minute. The number of words correctly recalled was determined at each testing.

The other cognitive function task assessed reaction times in an auditory vigilance test. It required the patient to discriminate target pips (pitch, 1200 Hz; duration, 60 milliseconds; intensity, 64 dB sound pressure level; $P = .1$), which were randomly interspersed among frequent standard pips of lower pitch (800 Hz). Each task sequence contained about 400 pips, presented with interstimulus intervals randomly varying between 1000 and 3000 milliseconds. The patient had to press a button with the thumb of the dominant hand as quickly as possible whenever he/she recognized a target pip. In addition to reaction time, omissions and false responses (button press after a standard pips) were recorded. However, the number of these types of responses was too small to yield any conclusive information. Therefore, respective data are not presented here.

2.6. Statistical analysis

Data are reported as mean ± SEM. Statistical analysis was generally based on analyses of variance including the repeated measure factor “hypo” for effects of hypoglycemia with reference to baseline measurements. Analyses also included the between-subject factor “sex” for differences between women and men. In these analyses the interaction term *hypo by sex* indicates differences in the response to hypoglycemia between sexes, provided that there are no differences during the baseline between sexes. Regarding the hormone data, the analysis of variance included all

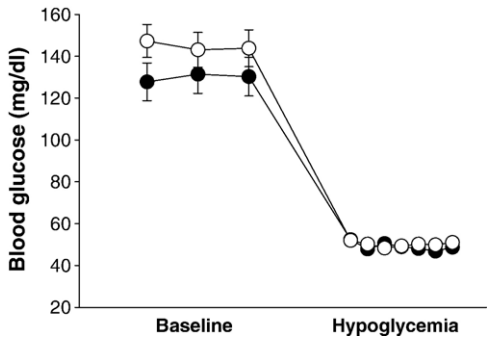

Fig. 1. Mean ± SEM blood glucose concentration in 15 women (open circles) and 15 men (black circles) with T2DM during a 30-minute baseline period and a 30-minute hypoglycemic plateau.

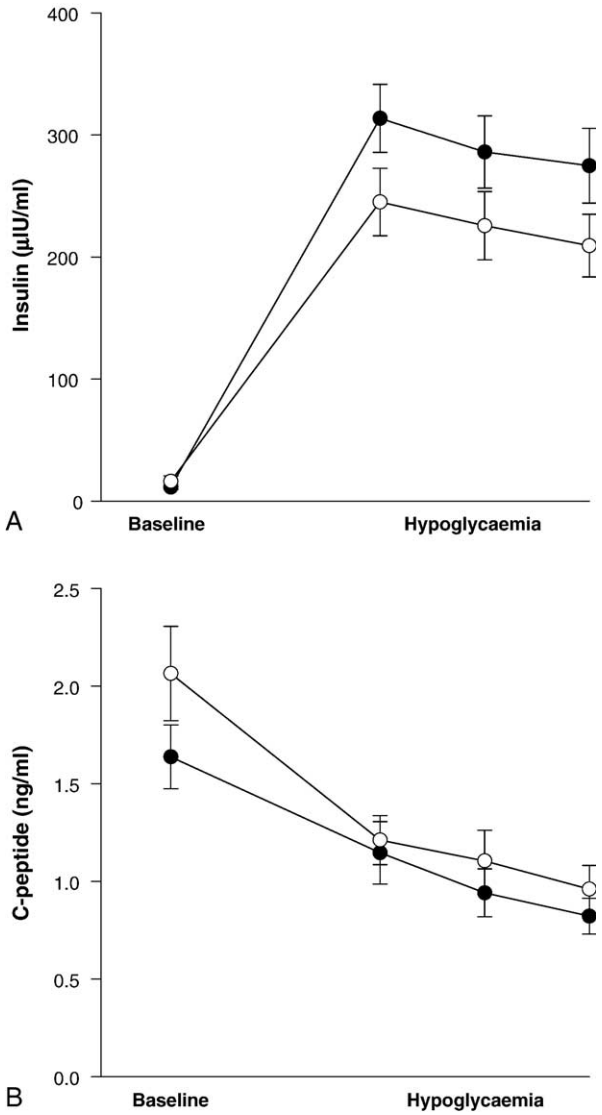

Fig. 2. Mean ± SEM serum insulin (A) and C-peptide (B) concentrations in 15 women (open circles) and 15 men (black circles) with T2DM during a baseline period and a 30-minute hypoglycemic plateau.

values measured during the clamps. In addition, we calculated the increase of each counterregulatory hormone by subtracting the baseline values from the values obtained in the end of hypoglycemia. For pairwise comparisons, unpaired Student *t* test was used. Statistical power calculation revealed that a sample size of 15 subjects in each group provided a power of 0.84 to detect a 100 pg/mL difference between groups in the peak epinephrine response to hypoglycemia assuming a within-group SD of 90 pg/mL. A *P* value of less than .05 was considered significant.

3. Results

3.1. Blood glucose, serum insulin, and C-peptide

Averaged baseline blood glucose levels were slightly higher in women than in men (145 ± 8 vs 130 ± 9 mg/dL), but this difference did not reach any significance (P = .231). In addition, the decrement in blood glucose levels from baseline to the hypoglycemic plateau did not differ significantly between women and men (78 ± 9 vs 92 ± 9 mg/dL; P = .291). The target blood glucose level of 50 mg/dL was first reached after 60 ± 7 minutes in women and after 47 ± 8 minutes in men (P = .203). During the 30-minute hypoglycemic plateau, blood glucose values were well comparable between the sexes (Fig. 1). Moreover, nadir glucose levels as defined by the minimum blood glucose level in each patient during the clamp did not differ between women and men (44.1 ± 1.1 vs 43.7 ± 1.1 mg/dL; P = .797).

Baseline serum insulin as well as C-peptide levels did not significantly differ between women and men (insulin, 18.7 ± 4.9 vs 11.4 ± 1.8 μU/mL, P = .155; C-peptide, 2.1 ± 0.2 vs 1.6 ± 0.2 ng/mL, P = .155). During insulin infusion, serum insulin levels reached steady-state levels of 226 ± 26 μU/mL in women and 291 ± 28 μU/mL in men (P = .102; Fig. 2A). In response to hypoglycemia, serum C-peptide levels decreased (P < .001) in a similar pattern in women and men reaching comparable nadir values of 0.96 ± 0.12 and 0.82 ± 0.09 ng/mL, respectively (P = .374; Fig. 2B).

3.2. Counterregulatory hormones and blood pressure

Baseline values and responses to hypoglycemia of counterregulatory hormones are summarized in Table 2. Plasma epinephrine levels were comparable between women and men at baseline (P = .380). In response to hypoglycemia, plasma epinephrine levels significantly increased (P < .001), but without any differences in this increase between women and men (P = .327; Fig. 3A).

Table 2
Mean ± SEM baseline hormone levels and counterregulatory responses to hypoglycemia

Variable	Women (n = 15)	Men (n = 15)	*P*
Epinephrine (pg/mL)			
Baseline	30.6 ± 10.5	42.6 ± 8.3	.380
Increase	147.7 ± 35.7	192.6 ± 57.5	.513
Norepinephrine (pg/mL)			
Baseline	408.9 ± 58.2	304.3 ± 29.4	.120
Increase	92.3 ± 33.4	123.1 ± 39.3	.554
Corticotropin (pg/mL)			
Baseline	14.6 ± 2.3	19.6 ± 2.9	.186
Increase	35.6 ± 14.6	29.5 ± 10.7	.739
Cortisol (μg/dL)			
Baseline	12.5 ± 1.5	14.6 ± 1.2	.276
Increase	5.9 ± 2.8	5.0 ± 1.5	.787
Growth hormone (ng/mL)			
Baseline	0.19 ± 0.05	0.99 ± 0.47	.107
Increase	2.95 ± 0.91	6.61 ± 2.66	.203
Glucagon (pg/mL)			
Baseline	177.9 ± 26.8	148.1 ± 20.4	.385
Decrease	−28.6 ± 12.2	−13.3 ± 15.6	.444

The increase in counterregulatory hormone was defined as the difference of the value obtained at the end of the hypoglycemia and the baseline value. For pairwise comparisons, Student *t* test was used.

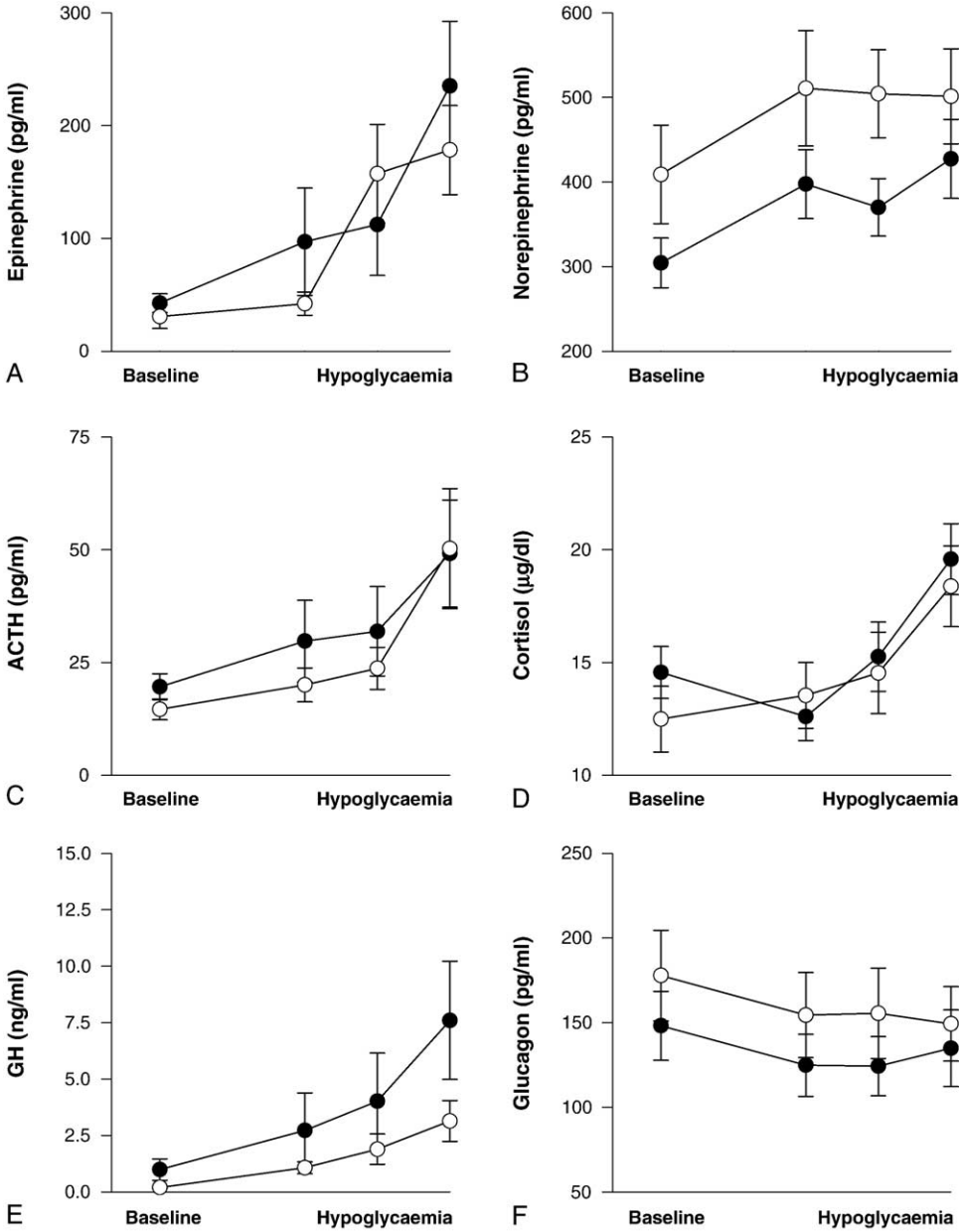

Fig. 3. Mean ± SEM levels of epinephrine (A), norepinephrine (B), ACTH (C), cortisol (D), growth hormone (E), and glucagon (F) in 15 women (open circles) and 15 men (black circles) with T2DM during a baseline period and a 30-minute hypoglycemic plateau.

Plasma norepinephrine levels tended to be higher in women than in men at baseline and throughout the clamp experiment (Fig. 3B), although these differences did not reach significance (P = .120 and .109, respectively). During hypoglycemia, plasma norepinephrine levels increased (P < .001), again with this increase being similar in women and men (P = .611).

Neither plasma corticotropin nor serum cortisol levels differed during baseline period between women and men (P > .185 for both comparisons). In addition, the hypoglycemia-induced increase of these hormones (both P < .007 for the increase) did not differ between sexes (P > .580 for both comparisons; Fig. 3C and D).

Basal growth hormone levels were comparable among women and men (P = .107). The hypoglycemia-induced increase of this hormone (P < .001) was somewhat, but nonsignificantly, weaker in women than in men (P = .256; Fig. 3E). Plasma glucagon levels did not differ during the baseline period (P = .385) nor did changes during the clamp differ between the 2 sexes (P = .604; Fig. 3F). Overall plasma glucagon levels slightly but significantly decreased during hypoglycemia (P = .014).

In response to hypoglycemia, systolic blood pressure increased from 145 ± 6 to 156 ± 7 mm Hg in women and from 154 ± 9 to 167 ± 10 mm Hg in men (P = .010 for the increase), whereas diastolic blood pressure did not

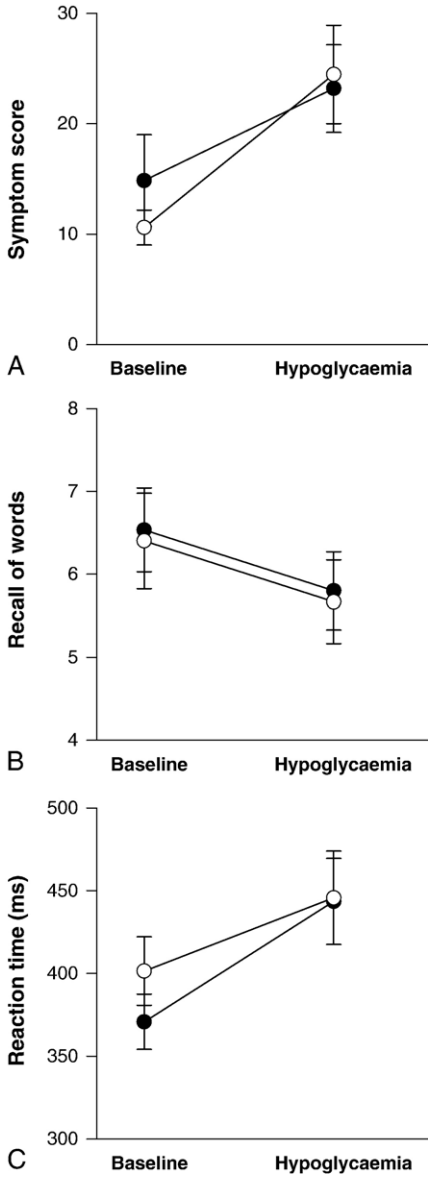

Fig. 4. Mean ± SEM symptom score (A), number of recalled words on a short-term memory task (B), and reaction time on an auditory attention task (C) in 15 women (open circles) and 15 men (black circles) with T2DM during a baseline period and a 30-minute hypoglycemic plateau.

significantly change during the clamp experiment (P = .407). The increase in systolic blood pressure showed no significant difference between sexes (P = .741).

3.3. Symptoms and neurocognitive functions

Averaged total symptom score increased during hypoglycemia (P < .001), but without any differences in this increase between women and men (P = .370; Fig. 4A). Separate analysis of autonomic and neuroglycopenic symptoms revealed similar results, with the increases in symptom scores during hypoglycemia being similar in women and men (P = .586 for autonomic and P = .248 for neuroglycopenic symptoms).

Recall performance of words tended to deteriorate during hypoglycemia (P = .091), with this deterioration being identical in women and men (P = 1.0; Fig. 4B). Reaction time on the auditory vigilance task significantly increased during hypoglycemia (P < .001), again without any difference in this increase between sexes (P = .350; Fig. 4C).

3.4. Sex hormones

According to their postmenopausal state, women had significantly higher serum gonadotropin levels than men (LH, 23.5 ± 2.4 vs 5.8 ± 1.3 mU/mL, P < .001; FSH, 46.3 ± 9.2 ± 2.6 mU/mL, P < .001), whereas serum estradiol levels were distinctly lower in women (18.7 ± 2.6 vs 30.6 ± 1.6 pg/mL; P < .001).

4. Discussion

In the present study, we compared hormonal, subjective, and neurocognitive responses to a standardized 30-minute steady-state hypoglycemia at a level of 50 mg/dL between postmenopausal women, not receiving an HRT, and men with T2DM. Although hypoglycemia induced profound changes in all parameters, none of these responses significantly differed between sexes. Thus, in the postmenopausal state, female patients with T2DM showed comparable responses to hypoglycemia compared with age-matched T2DM men.

It could be argued that the variability of responses to hypoglycemia was rather large, and, therefore, statistical power to detect differences between sexes was weak. Other variables such as glycemic control, diabetes duration, or diabetes therapy likely have contributed to the variability of response to hypoglycemia [7,9]. To avoid a systemic confounding influence of these factors on our results, we carefully matched the 2 groups of patients for all of these variables. In addition, we have studied a considerable number of patients, being 15 in each group, to increase statistical power to safely detect clinically relevant differences. However, it should be kept in mind that in the clinical situation the prevalence of potentially influencing factors likewise shows a great variability across patients with T2DM. On this background, it appears justified to conclude upon the present data that sex does not represent a major factor influencing hormonal, subjective, and neurocognitive responses to hypoglycemia in this group of patients.

Some possible limitations of the present study should be pointed out. First, the clamp procedure included only a single-step hypoglycemic plateau. This approach did not allow us to define glycemic thresholds for responses of respective parameters [18]. Thus, it could be possible that these glycemic thresholds differ between sexes. However, a previous study [19] directly addressing this issue in healthy subjects indicated that differential sex responses to hypoglycemia are due to alterations in central nervous system drive and not in glycemic thresholds, which renders this

possibility unlikely. Second, the hypoglycemia in our study was kept rather short, that is, 30 minutes, for ethical reasons to reduce the potentially harmful stress to our patients to a minimum. However, during this short-term hypoglycemia, counterregulatory hormone responses may not have reached a steady state, so that it cannot be completely ruled out that with more prolonged hypoglycemia, counterregulatory responses become different between postmenopausal women and men with T2DM. A single short bout of hypoglycemia may be basically a stimulus too weak to safely detect more discrete differences in hormonal counterregulation.

Serum insulin levels were on average higher in men than in women during the clamp, although this difference did not approach significance. Potentially, this difference could have affected counterregulatory responses to hypoglycemia because there is convincing evidence that insulin increases hormonal counterregulation [20-24]. However, based on an increasing influence of insulin, higher insulin levels in men are expected to enhance counterregulatory responses to hypoglycemia, thereby aggravating rather than diminishing differences in the response between women and men. Thus, it appears unlikely that any difference in circulating insulin levels biased our results toward reducing a potential difference in hormonal counterregulation between the sexes.

The present results may be taken to support a concept recently proposed by Sandoval et al [14] that estrogens represent an important factor for sex-specific differences in counterregulatory responses to hypoglycemia that have been found rather consistently in younger subjects. The women in our study were all in a postmenopausal state, and serum estradiol levels in these women were even lower than in the men. Thus, the failure in our study to detect any differences in hypoglycemia counterregulation between women and men could well be related to the fact that our postmenopausal women are characterized by distinctly lower blood concentrations of estradiol, compared with the younger premenopausal women tested in previous studies. Because we did not include here premenopausal women or those on estrogen replacement therapy as a control group, this hypothesis remains speculative.

At first glance, the decrease in glucagon levels during the hypoglycemic clamp represents a surprising finding that could support the previous notion that the glucagon response to hypoglycemia is impaired in insulin-treated patients with T2DM [7]. However, it should be noted that we infused insulin at a rather high rate (2.5 mU/kg per minute), thereby inducing marked hyperinsulinemia, to assure that hypoglycemia would be safely induced even in patients with a high degree of insulin resistance. Even at lower levels, hyperinsulinemia has been convincingly shown to attenuate the glucagon response to hypoglycemia and to decrease circulating glucagon levels during euglycemia [25]. On this background, the decrease in glucagon during hypoglycemia found in the present study may simply reflect the inhibitory effect of insulin on pancreatic α-cell glucagon secretion, which could have overridden the stimulatory influence of hypoglycemia.

In summary, based on the present results it appears justified to conclude that in contrast to previous observations in young and healthy subjects with or without T1DM, in the postmenopausal state, sex does not represent an important determinant of hormonal counterregulation to hypoglycemia in patients with T2DM. In addition, in these patients, sex appears to have no major influence on the perception of symptoms and the degree of neurocognitive dysfunction during hypoglycemia. These findings could explain the lack of any epidemiological evidence of differing rates of incidence rates for severe hypoglycemic episodes between women and men with T2DM. However, our data do not rule out a potentially harmful influence of estrogens on the risk of hypoglycemia. Therefore, it might be advisable in future epidemiological studies on this issue to more closely assess the menstrual state and the presence of hormone replacement therapy.

Acknowledgment

We thank Christiane Otten and Kamila Chara for their expert and invaluable laboratory assistance and Anja Otterbein for her organizational work.

This study was supported in part by a grant from the Germany Diabetes Society (Deutsche Diabetes Gesellschaft) to BS.

References

[1] The DCCT Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group. Am J Med 1991;90:450-9.

[2] UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998;352:854-865.

[3] Veneman TF, Erkelens DW. Clinical review 88: hypoglycemia unawareness in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1997;82:1682-4.

[4] Hepburn DA, MacLeod KM, Pell AC, et al. Frequency and symptoms of hypoglycaemia experienced by patients with type 2 diabetes treated with insulin. Diabet Med 1993;10:231-7.

[5] Banarer S, Cryer PE. Hypoglycemia in type 2 diabetes. Med Clin North Am 2004;88:1107-11.

[6] Heller SR. What we know about counterregulation in type 2 diabetes. Diabetes Nutr Metab 2002;15:372-5.

[7] Segel SA, Paramore DS, Cryer PE. Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. Diabetes 2002;51:724-33.

[8] Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic response to, symptoms of, and defense against subsequent hypoglycemia. J Clin Invest 1993;91:819-28.

[9] Korzon-Burakowska A, Hopkins D, Matyka K, et al. Effects of glycemic control on protective responses against hypoglycemia in type 2 diabetes. Diabetes Care 1998;21:283-90.

[10] Davis SN, Fowler S, Costa F. Hypoglycemic counterregulatory responses differ between men and women with type 1 diabetes. Diabetes 2000;49:65-72.

[11] Davis SN, Shavers C, Costa F. Gender-related differences in counter-regulatory responses to antecedent hypoglycemia in normal humans. J Clin Endocrinol Metab 2000;85:2148-57.
[12] Diamond MP, Jones T, Caprio S, et al. Gender influences counter-regulatory hormone responses to hypoglycemia. Metabolism 1993; 42:1568-72.
[13] Amiel SA, Maran A, Powrie JK, et al. Gender differences in counterregulation to hypoglycaemia. Diabetologia 1993;36:460-4.
[14] Sandoval DA, Ertl AC, Richardson MA, et al. Estrogen blunts neuroendocrine and metabolic responses to hypoglycemia. Diabetes 2003;52:1749-55.
[15] Veneman T, Mitrakou A, Mokan M, et al. Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia. Diabetes 1993;42:1233-7.
[16] Fruehwald-Schultes B, Born J, Kern W, et al. Adaptation of cognitive function to hypoglycemia in healthy men. Diabetes Care 2000; 23:1059-66.
[17] Schultes B, Kern W, Oltmanns K, et al. Differential adaptation of neurocognitive brain functions to recurrent hypoglycemia in healthy men. Psychoneuroendocrinology 2005;30:149-61.
[18] Mitrakou A, Ryan C, Veneman T, et al. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. Am J Physiol 1991;260:E67-E74.
[19] 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 2000;279: E1054-63.
[20] Davis SN, Shavers C, Collins L, et al. Effects of physiological hyperinsulinemia on counterregulatory response to prolonged hypoglycemia in normal humans. Am J Physiol 1994;267: E402-10.
[21] Davis SN, Goldstein RE, Jacobs J, et al. The effect of differing insulin levels on the hormonal and metabolic response to equivalent hypoglycemia in normal humans. Diabetes 1993;42:263-72.
[22] Davis SN, Dobbins R, Tarumi C, et al. Effects of differing insulin levels on response to equivalent hypoglycemia in conscious dogs. Am J Physiol 1992;263:E688-95.
[23] Fruehwald-Schultes B, Kern W, Deininger E, et al. Protective effect of insulin against hypoglycemia-associated counterregulatory failure. J Clin Endocrinol Metab 1999;84:1551-7.
[24] Fisher SJ, Bruning JC, Lannon S, et al. Insulin signaling in the central nervous system is critical for the normal sympathoadrenal response to hypoglycemia. Diabetes 2005;54:1447-51.
[25] Liu D, Moberg E, Kollind M, et al. A high concentration of circulating insulin suppresses the glucagon response to hypoglycemia in normal man. J Clin Endocrinol Metab 1991;23:1123-8.