Acta Diabetol (1998) 35: 1–8

ORIGINAL

K. Howorka · G. Heger · A. Schabmann · F. Skrabal
J. Pumprla

Weak relationship between symptom perception and objective hypoglycaemia-induced changes of autonomic function in hypoglycaemia unawareness in diabetes

Received: 4 July 1997 / Accepted in revised form: 27 November 1997

Abstract To assess the relationship between symptom perception and neurophysiological characteristics in hypoglycaemia unawareness, we investigated the awareness of symptoms, objective changes of autonomic function and counter-regulatory neuroendocrine responses to hypoglycaemia in intensively treated type I (insulin-dependent) diabetic patients with different degrees of hypoglycaemia unawareness. Hypoglycaemia (venous plasma glucose below 2.2 mmol/l) was induced with an intravenous insulin bolus in subjects with a history of repeated severe hypoglycaemia and hypoglycaemia unawareness ($n = 10$) and in a comparable group with good awareness of hypoglycaemia ($n = 8$). Autonomic symptoms, selected parameters of autonomic function and counter-regulatory hormones were assessed serially. Although hypoglycaemia was more pronounced in unaware patients (1.6 vs 2.0 mmol/l, $P = 0.05$), their induced adrenaline response was markedly impaired (delta adrenaline: 1.25 ± 1.10 vs 2.55 ± 1.46 nmol/l, $P = 0.05$). Astonishingly, differences between both patient groups in the course of autonomic function changes did not reach the level of significance ($P = 0.35 – 0.92$), although the unaware group reported markedly fewer autonomic symptoms, both neurogenic ($P = 0.001$) and neuroglycopenic ($P = 0.04$) than the aware group. This study indicates that in hypoglycaemia unawareness even extensive changes in autonomic function are not sufficient for the perception of hypoglycaemia and confirms that the central nervous system plays an important role in the awareness of hypoglycaemia.

K. Howorka (✉) · G. Heger · J. Pumprla
Department of Biomedical Engineering and Physics,
University of Vienna, Allgemeines Krankenhaus, Leitstelle 4L,
Währinger Gürtel 18–20, A-1090 Vienna, Austria

A. Schabmann
Department of Applied and Clinical Psychology,
University of Vienna, Vienna, Austria

F. Skrabal
Teaching Hospital of the Karl Franzens University, Graz, Austria

Key words Hypoglycaemia unawareness · Autonomic function · Glucose counter-regulation · Adrenaline · Growth hormone

Introduction

The risk of severe hypoglycaemia is increased in approximately 12% [1] to 26% of type I diabetic patients who cannot perceive symptoms of hypoglycaemia [2, 3]. Hypoglycaemia unawareness has been attributed to defective glucose counter-regulation [4], possibly induced by previous iatrogenic hypoglycaemia under intensified insulin treatment [5, 6]. Meticulous prevention of hypoglycaemia then restored in part the symptoms and counter-regulatory response to hypoglycaemia [7–9]. In contrast to previous theories, hypoglycaemia unawareness unexpectedly showed only a slight relationship to the degree of autonomic neuropathy [10] or to the application of human insulin [11].

It has been shown that measures most commonly used to prevent severe hypoglycaemia, such as frequent blood glucose self-monitoring and carrying glucose for the oral treatment of hypoglycaemia, are not always effective in patients at higher risk for severe hypoglycaemia [1]. Our previous investigation in patients with severe hypoglycaemia unawareness demonstrated the crucial role of the central nervous system (early decrease in vigilance) during hypoglycaemia [12]. The aim of this study was therefore to investigate the relationship between the awareness of different symptoms and objectively assessed selected autonomic function changes including the neuroendocrine counter-regulatory response to hypoglycaemia. The participants of the study were intensively treated type I diabetic patients with and without a history of typical hypoglycaemia unawareness syndrome including episodes of unconsciousness. Our goal was to search for potentially more effective measures for diabetic patients at risk.

Patients and methods

The study was approved and monitored by the Ethical Advisory Committee of the School of Medicine, University of Vienna. All subjects gave written informed consent. The study was conducted in accordance with the applicable guidelines for good clinical practice and the latest version of the Declaration of Helsinki [13]. The team involved in patient care and rehabilitation and in conducting the study was approved for conformity with European quality management and assurance standards ISO 9001 [14]. Patients were informed that they could request an interruption of the investigation by intravenous glucose administration at any time.

Eligibility criteria and patient characteristics

From our clinical survey population of about 300 insulin-dependent patients undergoing functional insulin treatment with multiple insulin injections or CSII (continuous subcutaneous insulin infusion), we recruited two patient groups comparable in age, duration of diabetes, late complications, diabetes education received and matched as far as possible for glycosylated haemoglobin (HbA_{1c}) level (Table 1). Both groups differed in their history of severe hypoglycaemic episodes and hypoglycaemia perception status as assessed by standardised interview. Since a history of severe hypoglycaemia with unconsciousness strongly predicts the recurrence of severe hypoglycaemic episodes [2, 3, 15], we classified patients as previously described [12], as having a clinically relevant unawareness of hypoglycaemia (i.e. unaware), if they reported repeated episodes of severe hypoglycaemia with unconsciousness and a persistent loss of perceived symptoms with blood glucose levels below 3.3–2.8 mmol/l for at least 1 year. Patients were classified as without hypoglycaemia unawareness (i.e. aware), if they never required assistance for treating hypoglycaemia and reported a usual threshold of hypoglycaemia perception of 3.3 mmol/l. These criteria were chosen as severe episodes of hypoglycaemia predominantly occur in hypoglycaemia-unaware patients. Altogether, 18 patients including 10 with poor awareness and 8 with good awareness of hypoglycaemia were selected. Patients with severe diabetes complications, e.g. neuropathy, either sensomotoric or autonomic [16], proliferative retinopathy or microproteinuria above 20 μg/min, were excluded.

Table 1 Clinical characteristics of type I diabetic patients with hypoglycaemia unawareness and subject to severe hypoglycaemic episodes, and those with good awareness of hypoglycaemia and never requiring external assistance (with the exception of frequency of severe hypoglycaemia, there were no significant differences between groups; *FIT* functional insulin treatment with multiple injections; mean±SD)

	With hypoglycaemia unawareness	Without hypoglycaemia unawareness
n (M/F)	10 (8/2)	8 (3/5)
Age (years)	37 ± 12	33 ± 10
Diabetes duration (years)	19 ± 11	12 ± 6
Duration of FIT (years)	4.6 ± 2.9	4.3 ± 2.7
Glycosylated haemoglobin (HbA_{1c}; %)	6.7 ± 0.6	7.4 ± 1.3
Weight (kg)	68.5 ± 7.0	65.4 ± 7.5
Body mass index (BMI; kg/m^2)	22.7 ± 2.1	22.4 ± 3.1
Patients with no/non-proliferative retinopathy	8/2	6/2
Insulin requirement (U/day)	50 ± 6	50 ± 14
Reported number of previous hypoglycaemic episodes with unconsciousness[a]	15 (3–78)	0

[a] median (range)

Study procedures

In the 2 weeks preceding the study, frequent blood glucose monitoring including nocturnal measurements was performed to avoid hypoglycaemia. All values below 5.3 mmol/l were corrected by an immediate oral intake of glucose. At least two blood glucose self-measurements were performed during the night preceding the study; biochemical hypoglycaemia (values below 3.3 mmol/l) was an exclusion criterion, and in that case the study was postponed. Patients received functional insulin treatment consisting of basal replacement by two daily injections of long-acting insulin and prandial or correctional boluses of regular insulin as previously described [17, 18]. Following an overnight fast (patients were allowed to drink water until 6.00 a.m.), patients had the last possible hyperglycaemia correction at 6.00 a.m., with intramuscular regular insulin according to individual algorithms for blood glucose correction targeting for 5.6 mmol/l. Basal insulin dosage was kept unchanged. At 7.30 a.m., patients were admitted to the neurology outpatient department and then maintained in a supine position in a quiet room. An indwelling intravenous cannula was inserted into an antecubital vein in each arm, one for taking blood samples and the other for the insulin bolus and for the (optional) intravenous administration of glucose. The essential condition for initiating the study and inducing hypoglycaemia was the stability of blood glucose for at least 90 min (fluctuations of 1.1 mmol/l per h were accepted). If the initial basal blood glucose was outside the range of 3.9–7.0 mmol/l, the study was postponed for several days. Hypoglycaemia was induced, usually between 10.30 and 11.00 a.m., by intravenous insulin (bolus of 0.08 U per kg body weight; human Actrapid, Novo Nordisk A/S, Bagsvaerd, Denmark).

Physiological measurements

A serial assessment (every 10 min) was made for a total of 140 min of blood pressure (automatic auscultatory sphygmomanometer), symptom evaluation and cognitive performance. Skin temperature (assessed by multiple temperature sensors), skin impedance and electrocardiogram (ECG) were monitored continuously for the 140-min duration of the experiment.

Biochemical measurements and blood analysis

Plasma glucose was measured every 5 min at the bedside in duplicate using a Beckman Analyzer 2 (Palo Alto, Ca., USA). The blood glucose values were not revealed to the patient. Blood withdrawals for the examination of glucose counter-regulatory hormones were performed every 10 min. Plasma was separated immediately and stored at –70°C for hormonal analysis. HbA_{1c} was measured by high-performance liquid chromatography (HPLC) (range in non-diabetic subjects: 4.0%–6.0%), insulin antibodies by enzyme-linked immunosorbent assay (ELISA) and C-peptide by radioimmunoassay (RIA) (coefficients of variation for the lowest range below 10%; commercial kits). Adrenaline and noradrenaline were measured by HPLC [19]; glucagon, growth hormone (GH) and cortisol by RIA commercial kits of Pharmacia-Kabi, DPC and TDX ABBOTT, respectively. Intra-assay variation was below 5%.

Symptoms of hypoglycaemia

Symptoms of hypoglycaemia were categorised, modified after Towler [20], as neuroglycopenic (confusion, dizziness, tired/weak, difficulty thinking, visual disturbance, warmth), neurogenic (i.e. autonomic-adrenergic: pounding heart, shaky/tremors, nervous/ anxious; and autonomic-cholinergic: sweaty, hungry, tingling) and non-specific (nausea, headache, shivering/cold, other symptoms). Patients rated a list of symptoms presented in random order on a scale of 0 (absent) to 3 (present, very strong). The total symptom score was calculated by adding together the symptom scores of each category. At the beginning of the experiment, the patients were also asked to report immediately if they felt hypoglycaemic. Cognitive function

was estimated as previously described [12, 17, 21] using a simple multiplication test: patients were asked to perform easy calculations, e.g. to multiply a one-digit number by a two-digit number, such as 7×14=? Patients who gave an incorrect answer were asked to multiply a one-digit by a one-digit number, e.g. 7×9=? This simple test was scored as follows: 0=correct results within 15 s; 1=patient needs longer than 15 s for a correct result; 2=wrong result but patient is able to multiply a one-digit by a one-digit number, 3=patient is unable to multiply a one-digit by a one-digit number.

Statistical analysis

Statistical analysis was performed using the standard statistical packages (SAS V 6.07 SAS SPSS Institute, Cary, N. C., USA; V2.2 SPSS, Chicago, Ill., USA). Results are expressed as mean±SEM, unless otherwise indicated. Variables that were not normally distributed were expressed as median values and analysed by the Mann Whitney U-test. Analysis of variance with repeated measurement design (general linear model) was used to evaluate differences between groups for changes in various parameters over time (two-way interaction group×time). Post-hoc tests were performed to compare single levels of increase/decrease of symptoms between groups. To show a possible relationship between symptoms and other variables, simple Pearson correlations were computed.

Results

Plasma glucose levels prior to hypoglycaemia induction were stable and did not differ statistically in the two patient groups (Fig. 1; time –20: $P = 0.57$; time zero $P = 0.25$) and were only slightly above the normoglycaemic range in non-diabetic persons [22]. The nadir of blood glucose was lower ($P = 0.045$) in the unaware patients (Table 2). However, despite the differences in the nadirs, the course of plasma glucose in both groups revealed no statistical differences. All patients counter-regulated spontaneously.

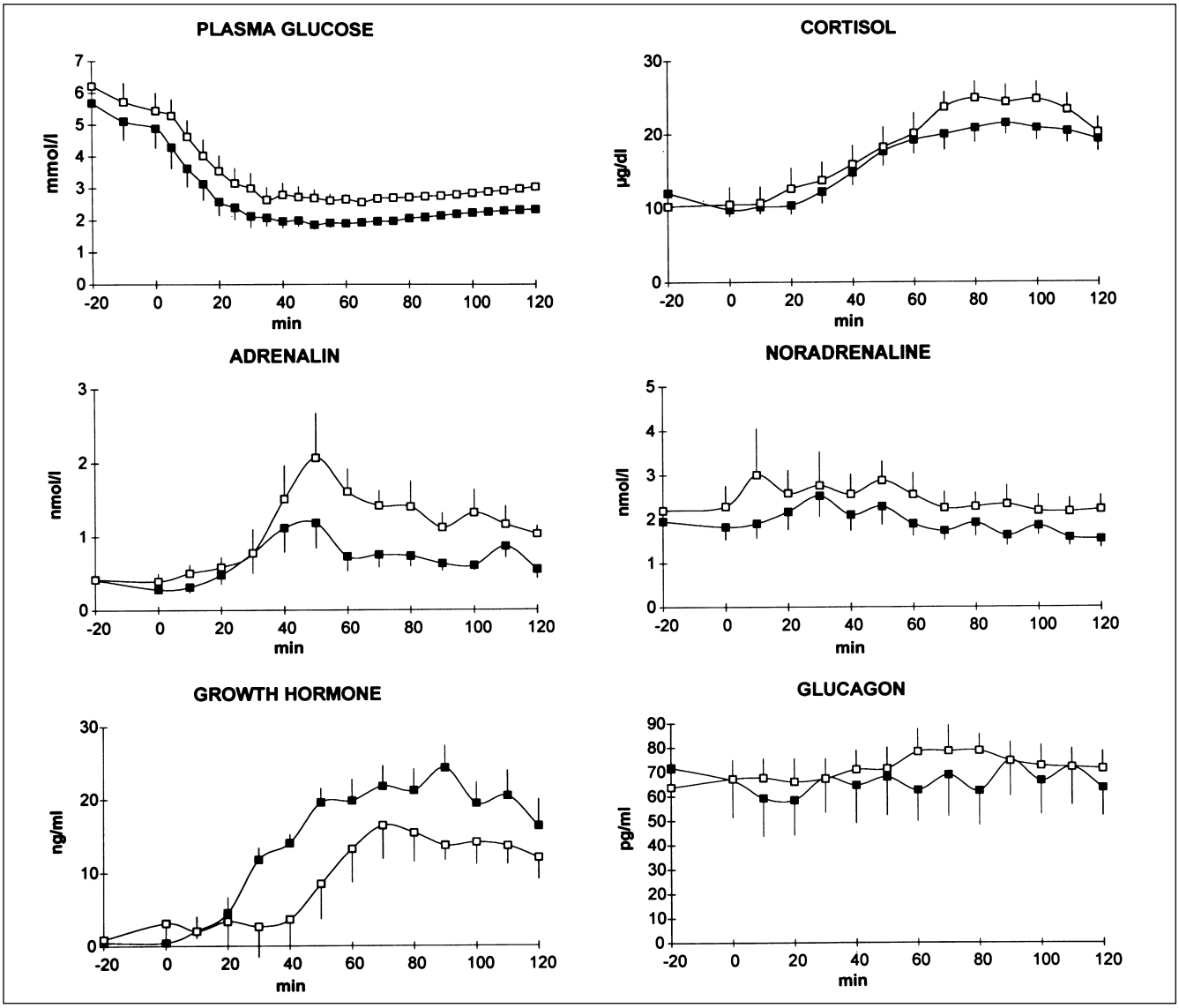


Fig. 1 Changes in plasma glucose and in counter-regulatory hormones in response to insulin-induced hypoglycaemia after the same hypoglycaemic stimulus in type I (insulin-dependent) diabetic patients with (*solid symbols*) and without (*open symbols*) hypoglycaemia unawareness

Table 2 Activation of counter-regulatory hormones and autonomic function changes during hypoglycaemia in patients with and without hypoglycaemia unawareness

	Unaware (n=10)	Aware (n=8)	P
Plasma glucose (PG)			
Basal (mmol/l)	4.48 ± 0.63	5.53 ± 0.57	NS
Minimum (mmol/l)	1.58 ± 0.16	2.03 ± 0.12	0.047
t (minimum) (min)	42.5 ± 7.1	47.8 ± 8.63	NS
Posthypoglycaemic glucose rise ([mmol/l]/min)	0.015 ± 0.002	0.021 ± 0.002	0.047
Final PG (mmol/l)	2.4 ± 0.12	3.09 ± 0.12	0.001
Adrenaline			
Basal (nmol/l)	0.28 ± 0.05	0.4 ± 0.11	NS
Maximum (nmol/l)	1.53 ± 0.34	2.95 ± 0.51	0.029
Delta (nmol/l)	1.25 ± 0.35	2.55 ± 0.52	0.046
Growth hormone			
Basal (ng/ml)	0.46 ± 0.43	0.01 ± 0.01	NS
Maximum (ng/ml)	30.11 ± 2.89	20.7 ± 6.29	0.025
Delta (ng/ml)	29.65 ± 2.83	20.69 ± 2.23	0.03
Cortisol			
Basal (µg/dl)	9.8 ± 0.06	10.5 ± 2.37	NS
Maximum (µg/dl)	22.4 ± 1.74	26.6 ± 2.26	NS
Delta (µg/dl)	12.6 ± 1.8	16.1 ± 3.32	NS
Noradrenaline			
Basal (nmol/l)	1.82 ± 0.29	2.29 ± 1.48	NS
Maximum (nmol/l)	2.99 ± 0.38	3.73 ± 1.0	NS
Delta (nmol/l)	1.17 ± 0.22	1.44 ± 0.59	NS
Glucagon			
Basal (pg/ml)	66.4 ± 15.2	64.9 ± 7.7	NS
Maximum (pg/ml)	80.8 ± 13.7	84.2 ± 8.8	NS
Delta (pg/ml)	14.4 ± 4.3	19.3 ± 3.3	NS
Heart rate			
Basal	67.9 ± 2.2	65.7 ± 2.7	NS
Maximum	80.3 ± 1.9	88.1 ± 3.3	NS
Delta	12.4 ± 2.5	22.5 ± 3.3	0.02
Forehead temperature			
Basal	33.8 ± 0.3	34.4 ± 0.2	NS
Minimum	32.7 ± 0.3	33.4 ± 0.3	NS
Delta	1.1 ± 0.3	1.0 ± 0.2	NS
Abdomen temperature			
Basal	34.2 ± 0.3	34.7 ± 0.3	NS
Minimum	33.2 ± 0.7	33.3 ± 0.6	NS
Delta	1.0 ± 0.5	1.4 ± 0.4	NS
Blood pressure			
RR syst (mmHg)			
Basal	118.0 ± 4.3	118.1 ± 5.7	NS
Maximum	130.5 ± 5.2	137.0 ± 8.1	NS
Delta	12.5 ± 3.8	19.6 ± 4.1	NS
RR diast			
Basal	79.0 ± 3.3	86.5 ± 3.3	NS
Minimum	72.8 ± 2.7	75.1 ± 4.6	NS
Delta	6.2 ± 2.7	11.3 ± 2.6	NS

The adrenaline response (Fig. 1) was significantly lower in the hypoglycaemia unaware group (delta adrenaline: $P = 0.046$) vs aware patients (Table 2). The GH response was more pronounced ($P = 0.03$) in this group and appeared somewhat earlier ($P = 0.09$) in comparison with the group of aware patients (Fig. 1). Differences between groups were found for the course of both adrenaline ($P < 0.001$) and growth hormone ($P < 0.001$). Cortisol, noradrenaline and glucagon values did not differ statistically in either patient group.

Both patient groups showed different behaviour patterns of symptom perception (Fig. 2). More hypoglycaemia symptoms were reported by aware patients in all symptom categories. Overall differences between groups were assessed for adrenergic ($P = 0.05$), cholinergic ($P = 0.001$), all autonomic (i.e. adrenergic plus cholinergic; $P = 0.001$), neuroglycopenic ($P = 0.04$) and total ($P = 0.002$) symptom scores. A different course between groups ($P = 0.001$) was also found for cognitive performance scores. The multiplication test used to assess cognitive performance achieved a maximal value of 1 and above only in the unaware group at 40–70 min after the induction of hypoglycaemia. Cognitive impairment was inversely related to the perceived symptoms in the entire study population (unaware and aware groups combined, total symptom score: $r = -0.59$, $P = 0.009$; cholinergic symptom score: $r = -0.51$, $P = 0.03$; adrenergic symptom score: $r = -0.58$, $P = 0.01$; neuroglycopenic symptom score: $r = -0.38$, NS; non-specific symptom score: $r = -0.44$, NS).

Pulse rate, temperature and blood pressure changes (Fig. 3) and maximal responses to hypoglycaemia (Table 2) revealed no significant differences between the patient groups nor for the course of blood pressure, heart rate and skin temperature at different locations ($P = 0.35$ to $P = 0.92$). With the exception of a more pronounced increase of heart rate, unaware patients showed in general almost as extensive changes in autonomic function, e.g. temperature response and perspiration during hypoglycaemia, as their aware controls. However, despite this only slightly weaker and usually very pronounced autonomic reaction, the unaware patients did not perceive the associated symptoms. This phenomenon of the dissociation between assessed autonomic function changes and symptom perception is illustrated in Fig. 4, showing a typical example of the time course of investigation in two representative patients without and with hypoglycaemia unawareness.

As the objective autonomic function changes were related to reported symptom scores, a significant correlation was found only in the aware group, which was strongest for neuroglycopenic symptoms and a relative decrease in skin temperature on the forehead ($r = 0.77$, $P = 0.02$) and abdomen ($r = 0.68$, $P = 0.05$). No significant correlations were found in unaware patients. Due to technical problems, flawless assessment of skin impedance was possible only in a few cases (Fig. 4), and an artefact-free measurement could not be obtained in a representative number of patients.

Discussion

In contrast to the clamp studies reported by others, our study was performed by using a single injection of insulin to simulate the type of hypoglycaemia found in everyday life and to observe the physiological responses not only of

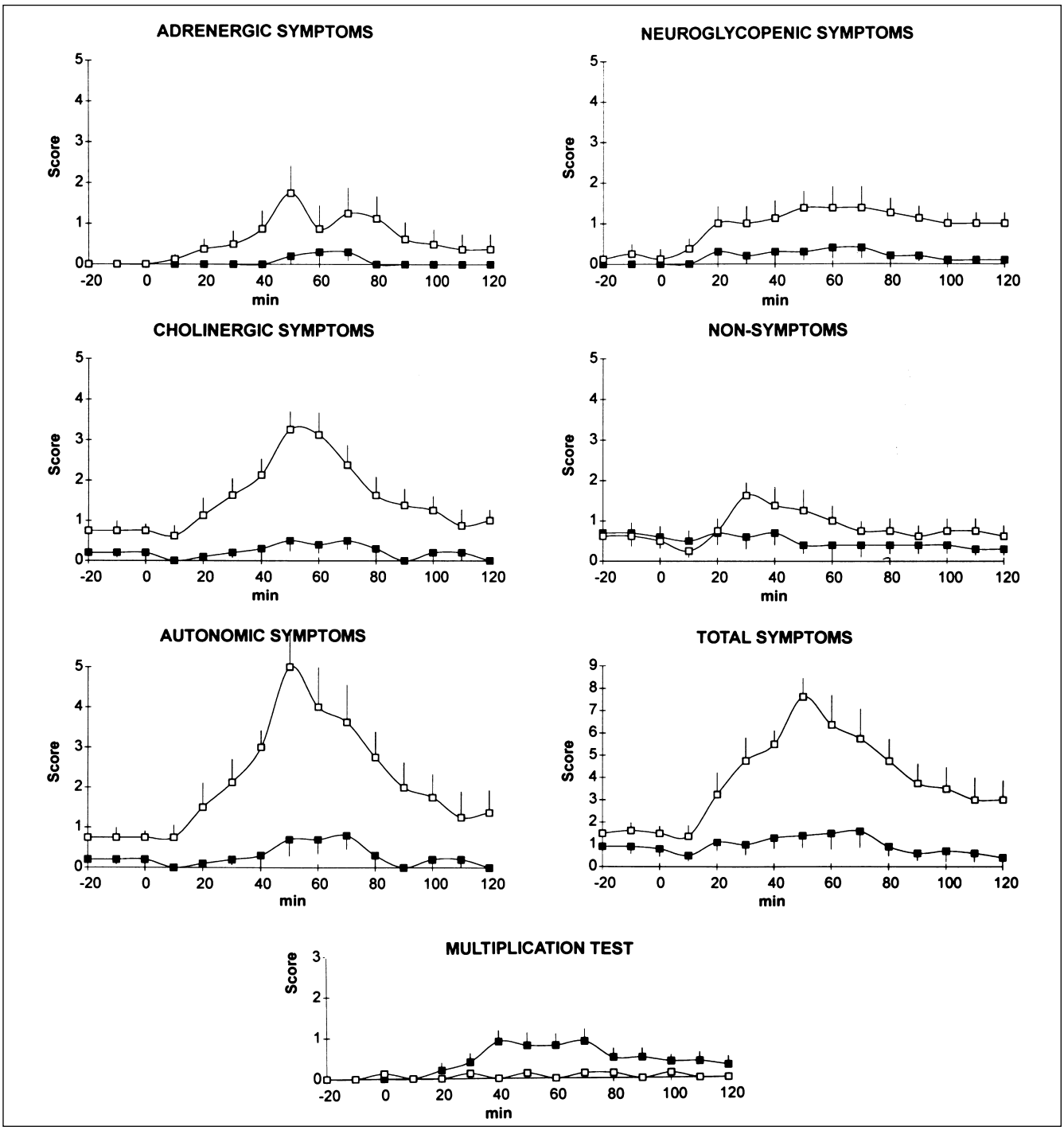


Fig. 2 Changes in different symptom scores during hypoglycaemia in patients with (*solid symbols*) and without (*open symbols*) hypoglycaemia unawareness

counter-regulatory hormones but also of the clinical consequences of hypoglycaemia. In our trial the plasma glucose course during hypoglycaemia was not different between patient groups. However, slight differences between plasma glucose nadirs illustrate the typical phenomenon of a stronger blood glucose lowering effect in the group with hypoglycaemia unawareness induced by the same stimulus as in the group without hypoglycaemia unawareness. For the purpose of our study, we believe that the bolus injection of insulin was appropriate, although further investigations of autonomic function changes under glucose clamp conditions are still required.

Patients with hypoglycaemia unawareness show a lower adrenaline response to hypoglycaemia [6, 9]. This finding was confirmed by our study. The preserved adrenaline response in aware patients may increase glucose production and, thereby, inhibit further lowering of blood glucose. This could be the possible explanation for the slightly

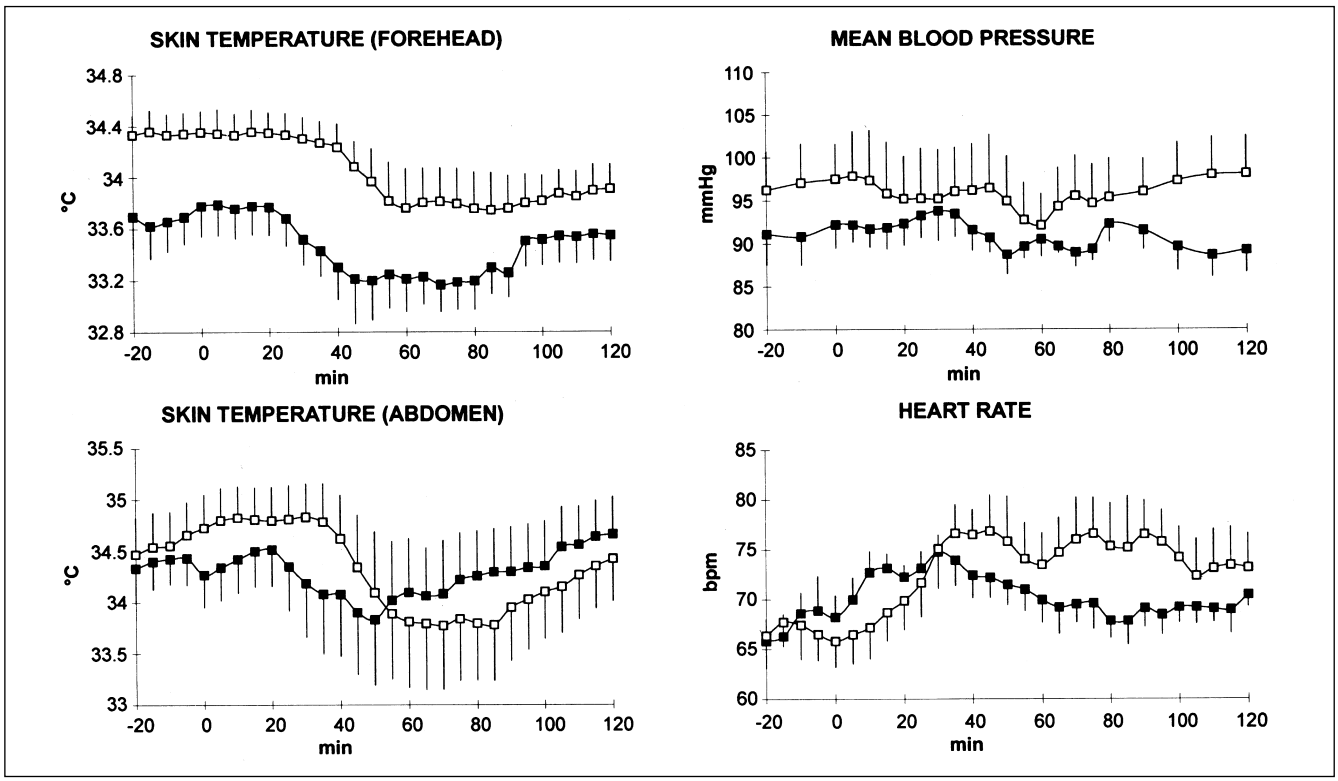


Fig. 3 Changes in autonomic function during hypoglycaemia in patients with (*solid symbols*) and without (*open symbols*) hypoglycaemia unawareness

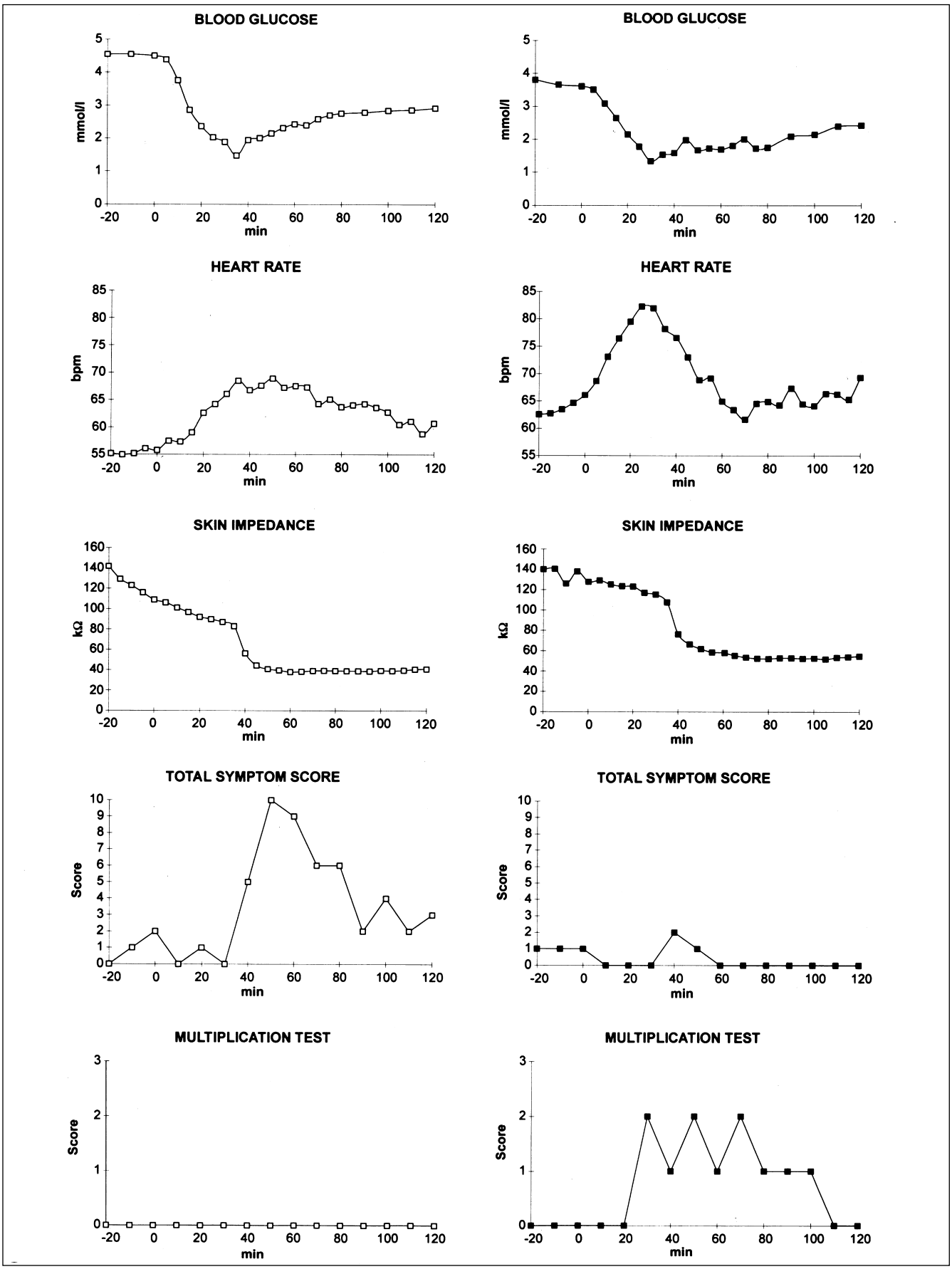
lower plasma glucose nadir in the unaware patient group. The lower plasma glucose levels in unaware patients may be responsible also for the increased GH secretion. However, the lower adrenaline response may have contributed to the higher GH levels by inducing less adrenaline-mediated inhibition of hypothalamic GH release via hypothalamic beta-2-adrenoceptors [23]. The higher adrenaline levels in aware patients may, therefore, suppress hypothalamic GH secretion. Our finding is in contrast to that of Frier et al. [24], who found an impaired GH response not only of other hypophyseal hormones, but also of GH during hypoglycaemia. If, however, adrenaline is important for suppressing GH secretion [23, 25], the lack of an adrenaline response makes an increased GH response plausible, as shown in our study.

The delayed glucose production induced by enhanced GH may contribute to the known brittleness in the unaware patient group. We did not find differences in noradrenaline, cortisol and glucagon responses to hypoglycaemia between both groups. As expected, patients unaware of hypoglycaemia and whose adrenaline increased less experienced fewer adrenergic symptoms, which confirmed previous findings [26, 27].

To our knowledge, this is the first reported study designed to relate the perceived symptoms to the real changes in autonomic function in hypoglycaemia unawareness. Indeed, it became clear that autonomic function changes were often present or only slightly weaker but were not recognised, and symptoms were not perceived in the unaware group. Objective measurements of autonomic reaction such as heart rate, skin temperature and perspiration measured as a decrease in skin impedance showed marked changes during hypoglycaemia in both groups. However, the perception of these warning symptoms in unaware patients was virtually absent, confirming the decisive role of the central nervous system for the perception of hypoglycaemia [12]. Theoretically, other alternative substrates for the brain like lactate or ketones could play a role in modifying the awareness of hypoglycaemia [28]. Under the conditions of our study, however, no differences in lactate concentrations between groups would be expected. Endogenous hyperketonaemia during insulin-induced hypoglycaemia proved not to contribute to brain metabolism and function [29].

Cognitive function was impaired markedly and earlier only in the unaware patient group, which might be related also to their slightly lower plasma glucose levels. Further investigations are necessary to determine whether the enhanced GH secretion is associated with the early vigilance

Fig. 4 A typical dissociation between autonomic function changes and symptom perception in hypoglycaemia unawareness. Comparison between subjective total symptom score and autonomic changes during hypoglycaemia in two representative patients (*left* without and *right* with hypoglycaemia unawareness): a decrease in skin impedance (i.e. perspiration) and an increase in heart rate were not associated with an increase in the symptom score in the patient with hypoglycaemia unawareness. Hypoglycaemia perception (total symptom score) reflects autonomic changes in the aware patient


BLOOD GLUCOSE
mmol/l
min
BLOOD GLUCOSE
mmol/l
min
HEART RATE
bpm
min
HEART RATE
bpm
min
SKIN IMPEDANCE
kΩ
min
SKIN IMPEDANCE
kΩ
min
TOTAL SYMPTOM SCORE
Score
min
TOTAL SYMPTOM SCORE
Score
min
MULTIPLICATION TEST
Score
min
MULTIPLICATION TEST
Score
min

decrease [12, 30] during hypoglycaemia in unaware patients.

In conclusion, our study has demonstrated only a weak relationship among perceived symptoms and autonomic function changes during hypoglycaemia in unawareness. Moreover, that which is essential for the development of new strategies in patient education – the cognitive deterioration in patients with hypoglycaemia unawareness – is inversely correlated to the increase of neurogenic (autonomic, cholinergic and adrenergic) symptoms. The hormonal counter-regulation, therefore, appears to be only partially responsible for symptom awareness, once more indicating the importance of the central nervous system [12].

The practical value of a simple calculation task for the early detection of hypoglycaemia and prevention of severe episodes [12, 17] should be investigated in programmes to prevent hypoglycaemia and restore symptom perception in patients with hypoglycaemia unawareness.

Acknowledgements This study was partly supported by the Fonds zur Förderung der wissenschaftlichen Forschung FWF, Vienna, Austria (Project S4909).

References

1. Howorka K, Grillmayr H, Waldhäusl W (1986) The incidence of severe hypoglycaemia during near-normoglycemic insulin substitution: the "hypoglycaemia-cluster" phenomenon. Diabetologia 29: 551A
2. DCCT Research Group (1991) Epidemiology of severe hypoglycaemia in the diabetes control and complications trial. Am J Med 90: 450–459
3. DCCT Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 32: 971–986
4. Cryer PE, Gerich JE (1985) Glucose counter-regulation, hypoglycaemia, and intensive insulin therapy in diabetes mellitus. N Engl J Med 313: 232–241
5. Cryer PE (1993) Hypoglycaemia begets hypoglycaemia in IDDM. Diabetes 42: 1691–1693
6. Davis MR, Mellmann M, Shamoon H (1992) Further defects in counter-regulatory responses induced by recurrent hypoglycaemia in IDDM. Diabetes 41: 1335–1341
7. Fanelli C, Epifano L, Rambotti A, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusanio F, Brunetti P, Bolli G (1993) Meticulous prevention of hypoglycaemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycaemia in intensively treated patients with short-term IDDM. Diabetes 42: 1683–1689
8. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, DiVincenzo A, Modarelli E, Ciofetta M, Lepore M, Annibale B, Torlone E, Perriello G, DeFeo P, Santeusanio E, Brunetti P, Bolli G (1994) Long-term recovery from unawareness, deficient counter-regulation and lack of cognitive dysfunction during hypoglycaemia, following institution of rational, intensive insulin therapy in IDDM. Diabetologia 37: 1265–1276
9. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA (1994) Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. Lancet 344: 283
10. Ryder R, Owens D, Hayes T, Ghatei M, Bloom S (1990) Unawareness of hypoglycaemia and inadequate hypoglycemic counter-regulation: no causal relation with diabetic autonomic neuropathy. Br Med J 301: 783–787
11. Patrick AW, Bodmer CW, Tieszen KL, White MC, Williams G (1991) Human insulin and awareness of acute hypoglycemic symptoms in insulin-dependent diabetes. Lancet 338: 528–532
12. Howorka K, Heger G, Schabmann A, Anderer P, Tribl G, Zeitlhofer J (1996) Severe hypoglycaemia unawareness is associated with an early decrease in vigilance during hypoglycaemia. Psychoneuroendocrinology 21: 295–312
13. Declaration of Helsinki (updated version 1989, Hong-Kong). Recommendations guiding medical physicians in biomedical research involving human subjects. http://www.sar.co.uk/helsinki.htm
14. Howorka K, Pumprla J, Kletschka G, Thoma H (1998) ISO 9001 – conformity in research, graduate education and rehabilitation. Biomed Technik (in press)
15. White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV (1983) Identification of type I diabetic patients at increased risk for hypoglycaemia during intensive therapy. N Engl J Med 308: 485–491
16. Ewing DJ, Martyn CN, Young RJ, Clarke BF (1985) The value of cardiovascular autonomic function tests: 10 years experience in diabetes. Diabetes Care 8: 491–498
17. Howorka K (1996) Functional insulin treatment. Principles, teaching approach and practice, 2nd edn. Springer, Berlin Heidelberg New York. German version: Funktionelle Insulintherapie. Lehrinhalte, Praxis und Didaktik, 4th edn. Springer, Berlin Heidelberg New York
18. Waldhäusl W (1986) Physiological basis of insulin treatment – clinical aspects. Diabetologia 29: 837–849
19. Boos KS, Wilmers B, Sauerbrey R, Schlimme E (1988) On-line sample processing and analysis of diol compounds in biological fluids. J Chromatogr 456: 93–104
20. Towler DA, Havlin CE, Craft S, Cryer P (1993) Mechanism of awareness of hypoglycaemia. Diabetes 42: 1791–1798
21. Meyer K, Landgren W (1980) TÜLUC Tübinger Luria Christenson. Neuropsychologische Untersuchungsreihe, Beltz-Test, Weinheim
22. Hepburn DA, Deary IJ, Frier BM, Patrick AW, Quinn JD, Fisher BM (1991) Symptoms of acute insulin-induced hypoglycaemia in humans with and without IDDM. Diabetes Care 14: 949–957
23. Blackard W, Heidingsfelder S (1968) Adrenergic receptor control mechanism for growth hormone secretion. J Clin Invest 47: 1407–1414
24. Frier BM, Fisher BM, Gray CE, Beastall GH (1988) Counter-regulatory hormonal responses to hypoglycaemia in type 1 (insulin-dependent) diabetes: evidence for diminished hypothalamic-pituitary hormonal secretion. Diabetologia 31: 421–429
25. Collu R, Fraschini F, Visconti P, Martini L (1972) Adrenergic and serotoninergic control of growth hormone secretion in adult male rats. Endocrinology 90: 1231–1237
26. Grimaldi A, Bosquet F, Davidoff P, Digy JP, Sachon C, Landault C, Thervet F, Zoghbi F, Legrand JC (1990) Unawareness of hypoglycaemia by insulin-dependent diabetes. Horm Metab Res 22: 90–95
27. Hepburn DA, Patrick AW, Brash HM, Thomson I, Frier BM (1991) Hypoglycaemia unawareness in type 1 diabetes: a lower plasma glucose is required to stimulate sympatho-adrenal activation. Diabetic Med 8: 934–945
28. Maran A, Cranston I, Lomas J, MacDonald I, Amiel SA (1994) Protection by lactate of cerebral function during hypoglycaemia. Lancet 343: 16–20
29. Fanelli C, Di Vincenzo A, Modarelli F, Lepore M, Ciofetta M, Epifano L, Pampanelli S, Brunetti P, Bolli GB (1993) Post-hypoglycaemic hyperketonaemia does not contribute to brain metabolism during insulin-induced hypoglycaemia in humans. Diabetologia 36: 1191–1197
30. Takahashi V, Kipnis DM, Daughaday WH (1968) Growth hormone secretion during sleep. J Clin Invest 47: 2079–2090