Insulin Absorption, Glucose Homeostasis, and Lipolysis in IDDM During Mental Stress

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Objective: To study the effects of mental stress on the absorption kinetics of insulin and on glucose homeostasis and lipolysis in insulin-dependent diabetes mellitus (IDDM). Research Design and Methods: Nine IDDM patients were exposed to the Stroop color word conflict test (CWT) during 40 min after injection of ^{125}I-labeled soluble human insulin (10 U) into the abdomen. Adipose tissue blood flow (^{133}Xe-clearance) was determined concomitantly to elucidate the importance of blood flow for insulin absorption during CWT. The effect of the CWT was followed by measurement of arterial levels of catecholamines and as blood pressure and heart-rate responses. Lipolysis was measured as arterial glycerol levels, and ketone body levels were monitored by determination by β-hydroxybutyrate. Results: Although insulin absorption (residual ^{125}I-radioactivity and plasma free insulin levels) and the arterial levels of glucose and β-hydroxybutyrate were not significantly changed by the CWT, arterial glycerol and norepinephrine levels and adipose tissue blood flow were approximately doubled, and epinephrine levels increased fourfold. Heart rate increased ~35 beats/min and mean blood pressure ~25 mmHg. Conclusions: The results suggest that intense mental stress of 40 min duration does not alter the absorption of subcutaneously injected insulin, glucose homeostasis, or ketone body levels in patients with IDDM, despite a considerable increase in blood flow and lipolysis.
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Metabolic decompensation is considered to occur frequently in diabetic patients during mental stress. Thus, increases in free fatty acid levels (1,2) and even ketoacidosis (3) have been described in connection with psychological stress. Different glycemic responses to psychological stress have been suggested. Both hyperglycemia (1) and hypoglycemia (2,4) and unaltered glucose homeostasis (5) have been reported.

Because the absorption of subcutaneously injected insulin is considered to be of major importance for glycemic control in insulin-dependent diabetic (IDDM) patients (6,7), we found it of interest to investigate whether insulin absorption is influenced by mental stress in IDDM, a question that has not been previously addressed. Mental stress can be evoked in a standardized way with the Stroop color word conflict test (CWT; 8). Extensive studies in healthy subjects have shown it to be a well-defined mental stress test of great potency (9–11).

The aim of this investigation was to study effects of the CWT on the absorption of subcutaneously injected insulin in IDDM. In addition, the study enabled us to reevaluate glucose homeostasis and lipolysis as well as ketone body levels and sympathoadrenal activity in IDDM patients during standardized mental stress.

RESEARCH DESIGN AND METHODS

Nine patients with IDDM who lacked endogenous insulin secretion (plasma C-peptide <0.05 nM) participated in the study. The individual characteristics of the subjects are given in Table 1. Two patients (no. 1 and 2) had background retinopathy (microaneurysms only)

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TABLE 1
Individual characteristics of diabetic patients

n	Sex (M/F)	Age (yr)	Body mass index (kg/m²)	Diabetes duration (yr)	Insulin dose (U · kg⁻¹ · day⁻¹)	HbA_{1c} (%)*
1	M	33	23.8	17	0.66	7.9
2	F	24	23.5	16	0.65	12.2
3	F	27	22.0	18	0.81	7.9
4	M	23	21.1	17	0.71	7.1
5	M	29	25.1	11	1.60	5.8
6	M	21	24.6	9	0.88	7.4
7	M	27	23.3	13	0.76	6.4
8	F	43	22.5	31	0.49	6.3
9	F	42	20.9	17	0.85	8.7
Means ± SE	5/4	30 ± 3	23.0 ± 0.5	17 ± 2	0.82 ± 0.10	7.7 ± 0.6

*Normal value <5%.

and two had intermittent proteinuria (no. 2 and 3). The parasympathetic nervous system was evaluated from the heart-rate variation during deep breathing (expiration-inspiration ratio, abnormal value <1.10; 12) and a Valsalva maneuver (Valsalva ratio, abnormal value <1.10; 13). The mean expiration-inspiration and Valsalva ratios were normal measuring 1.44 ± 0.09 (range 1.25–1.94) and 1.51 ± 0.08 (range 1.09–2.08), respectively. One patient (no. 8) showed an abnormal Valsalva ratio (1.09), but the expiration-inspiration ratio and the sympathetic nervous function tests were normal. The latter included the heart rate, intra-arterial blood pressure, and arterial plasma norepinephrine responses toward standardized tilt (14). Basal heart rate measured 62 ± 3 beats/min, arterial blood pressure 133 ± 4/67 ± 2 mmHg, and arterial plasma norepinephrine levels 1.25 ± 0.22 nM. At 6 min of tilt, the corresponding increases were 18 ± 2 beats/min, 3 ± 3/9 ± 2 mmHg, and 1.25 ± 0.22 nM, respectively. These responses were similar to those of a healthy control group previously studied under identical experimental conditions (15).

The patients were informed of the nature, purpose, and possible risks of the study before giving their informed consent to participate in the investigation, which was approved by the ethics committee at Huddinge Hospital.

The patients were admitted to the hospital the day before the study. The last subcutaneous injection of soluble insulin was given on the morning of admittance, whereafter an intravenous infusion of soluble insulin was started, aiming at a blood glucose concentration of 6–8 mM in the morning of each investigation. The insulin infusion lasted ~18 h. At the cessation of the insulin infusion, blood glucose measured 7.9 ± 1.0 and 8.6 ± 1.1 mM (NS) at 0700 on the test and control days, respectively. To clear the circulation from the infused insulin, the intravenous insulin infusion was discontinued 30 min before the experiments. Therefore, the basal glucose levels given in Fig. 2*A* differ somewhat from the goal. The experiments started at 0730 after an overnight fast. Smoking was prohibited. To reduce thyroid uptake of ^{125}I, potassium iodide was given orally.

Under local anesthesia, thin Teflon catheters were inserted into a brachial artery for blood sampling and blood pressure measurements and into a cubital vein for glucose infusions. To avoid hypoglycemia after the subcutaneous insulin injection, the glucose-clamp technique was used (16). Adipose tissue blood flow was determined with the ^{133}Xe-clearance technique with a tissue-blood partition coefficient of 10 ml/g (17). ^{133}Xe (0.1 ml, 40 kBq) was injected subcutaneously ~10 cm lateral to the umbilicus into the middle of the abdominal subcutaneous tissue after ultrasound determination (Interspec, Cardioscan, Conshohocken, PA) of the depth of the adipose tissue layer (18). Insulin absorption was assessed from the disappearance of ^{125}I-radioactivity by external γ-counting (19) and as appearance of plasma free immunoreactive insulin. Twenty minutes after the ^{133}Xe injection, 10 U radiolabeled soluble human insulin (20 kBq, Actrapid Human, Novo Copenhagen; 20) was injected subcutaneously into the contralateral side. The limitations associated with the determination of adipose tissue blood flow at a site contralateral to the insulin injection have been discussed elsewhere (21), and control experiments have shown similar responses of the ^{133}Xe elimination rates from ipsilateral and contralateral sites. The residual radioactivities of ^{125}I and ^{133}Xe were followed continuously by external γ-counting with attachable light-weight scintillation detectors (0.5 × 0.5 inch; LEAB, Mölnlycke, Sweden) fixed over the injection sites with elastic bandages. The detectors were coupled to a spectrometer (ND62 or ND600, Schaumburg, IL). Counts were accumulated during consecutive 60-s intervals.

After catheterization, the patients rested in a semirecumbent position. Sixty minutes after the subcutaneous insulin injection, mental stress was evoked for 40 min by a modified videotaped version of the Stroop CWT, which was run twice (8). The stress test involved presenting the subjects with a color word (green, red, yellow, and blue) written in an incongruent color on a

television screen, while another color word was spoken (in headphones) by a disturbing voice. The patients were asked to mark on a protocol the color in which the color word was written and ignore all the irrelevant information. The total number of presentations was 1260. The subjective feelings of stress, irritation, tiredness, and heart palpitations were rated on a visual analogue scale (presenting minimal to maximal experiences) before and after the stress. During the control day, the patients were studied resting quietly in the semirecumbent position. The order between the test and the control days was randomized. Before, during, and after the stress test, and during the control day, arterial blood samples were taken for the determination of glucose, free immunoreactive insulin, β-hydroxybutyrate, glycerol, and catecholamines at the time intervals indicated in Figs. 1 and 2. Arterial blood pressure (Siemens-Elema mingograph 62, Erlangen, Germany) and heart rate (1-lead ECG) were measured intermittently. The stress test greatly increased the feelings of stress and irritation. Also, the self-awareness of heart palpitations increased, but the feeling of tiredness was not affected.

Plasma free immunoreactive insulin was determined according to Heding (22) after immediate polyethylene glycol precipitation (23). Plasma glucose was analyzed by a glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Blood glycerol (24) and β-hydroxybutyrate (25) were measured by fluorimetric methods. Arterial plasma epinephrine and norepinephrine were analyzed by high-performance liquid chromatography with electrochemical detection (26).

Data analysis. An Apple II microcomputer (Cupertino, CA) was used to calculate the fractional disappearance rates of ^{133}Xe and ^{125}I with regression analysis of the natural logarithms of the counts accumulated over consecutive 20-min intervals. The residual ^{125}I-radioactivity in percentage of the initial value was also calculated. The area under the plasma free immunoreactive insulin curve was determined by trapezoidal integration. Adipose tissue vascular resistance (expressed in peripheral resistance units) was calculated by dividing the mean arterial blood pressure by adipose tissue flood flow.

Statistical analysis. Analysis of variance for repeated measurements and Student's *t* test for paired and unpaired observations were used where applicable. Results are presented as means ± SE. $P < 0.05$ was significant.

RESULTS

During the control day, the disappearance rate of ^{125}I-radioactivity increased significantly ($P < 0.05$) from the first to the last 20-min period. A similar increase occurred also during the stress day. No differences were found in insulin disappearance rates before, during, or after stress versus the control day (Fig. 1*A*). The residual radioactivities did not differ between days, as shown by the almost congruent curves and the values at the end of the stress test and on the control day being 65 ± 5 and 61 ± 3%, respectively (Fig. 1*B*).

Basal plasma free immunoreactive insulin levels were similar during the stress and control days (7 ± 3 and 6 ± 3 mU/L). After the subcutaneous insulin injection, the plasma free immunoreactive insulin levels did not differ significantly between the stress and control days (Fig. 1*C*). Also, the areas under the plasma immunoreactive insulin curves both before, during, and after the CWT were similar on the stress and control days (1007 ± 287 vs. 923 ±176, 984 ± 231 vs. 1124 ± 150, and 1629 ± 295 vs. 1796 ± 251 mU/L × min, respectively).

Basal arterial plasma glucose levels were similar on the stress and control days measuring 10.2 ± 0.8 and 10.6 ± 0.5 mM, respectively. The plasma glucose curves showed a similar course during the 2 study days and were not significantly affected by the stress (Fig. 2*A*). Five patients were given intravenous glucose for 29 ± 9 min during the stress day and three of these patients and two others received intravenous glucose for 26 ± 6 min during control (NS between days). The amounts of glucose infused on the stress and control days did not differ significantly, measuring 49.5 ± 18.4 vs. 44.0 ± 13.2 mg/kg, respectively.

On the stress day, blood glycerol levels decreased from an initial level of 70.9 ± 28.8 to 24.8 ± 2.0 μM immediately before the CWT 60 min after the subcutaneous insulin injection (Fig. 2*B*). These values were not significantly different from those obtained on the control day (78.7 ± 13.2 and 28.1 ± 6.3 μM, respectively). During the CWT, blood glycerol increased, reaching a maximal value of 48.6 ± 7.4 μM at 17 min of stress, which corresponds to an increase of ~90% vs. prestress levels and 120% vs. the control day ($P < 0.01$ and $P < 0.001$, respectively). Twenty minutes after the termination of the CWT, blood glycerol had returned to ~35 μM, which was not significantly different from the corresponding value on the control day. At 80 min after the stress, however, the glycerol level had returned to approximately the maximum level measured during CWT, and it was again significantly higher than on the control day ($P < 0.05$).

Blood β-hydroxybutyrate levels were obtained from eight patients. After insulin injection, the level decreased significantly, from 0 to 60 min during both study days ($P < 0.05$ for both). The magnitude of this decrease was similar on the CWT (−78.8 ± 98.9 μM) and control days (−130.9 ± 98.5 μM, NS). During the CWT, the β-hydroxybutyrate concentration fell continuously; the maximum decrease (−171.9 ± 80.7 μM) was not significantly different from the response on the control day.

Basal arterial plasma epinephrine and norepinephrine levels measured 0.22 ± 0.03 and 1.05 ± 0.14 nM, respectively. During the CWT, a fourfold increase in plasma epinephrine was found, whereas plasma norepinephrine levels were approximately doubled compared with the control day. Maximal epinephrine and norepinephrine levels were found at 3 min of stress,

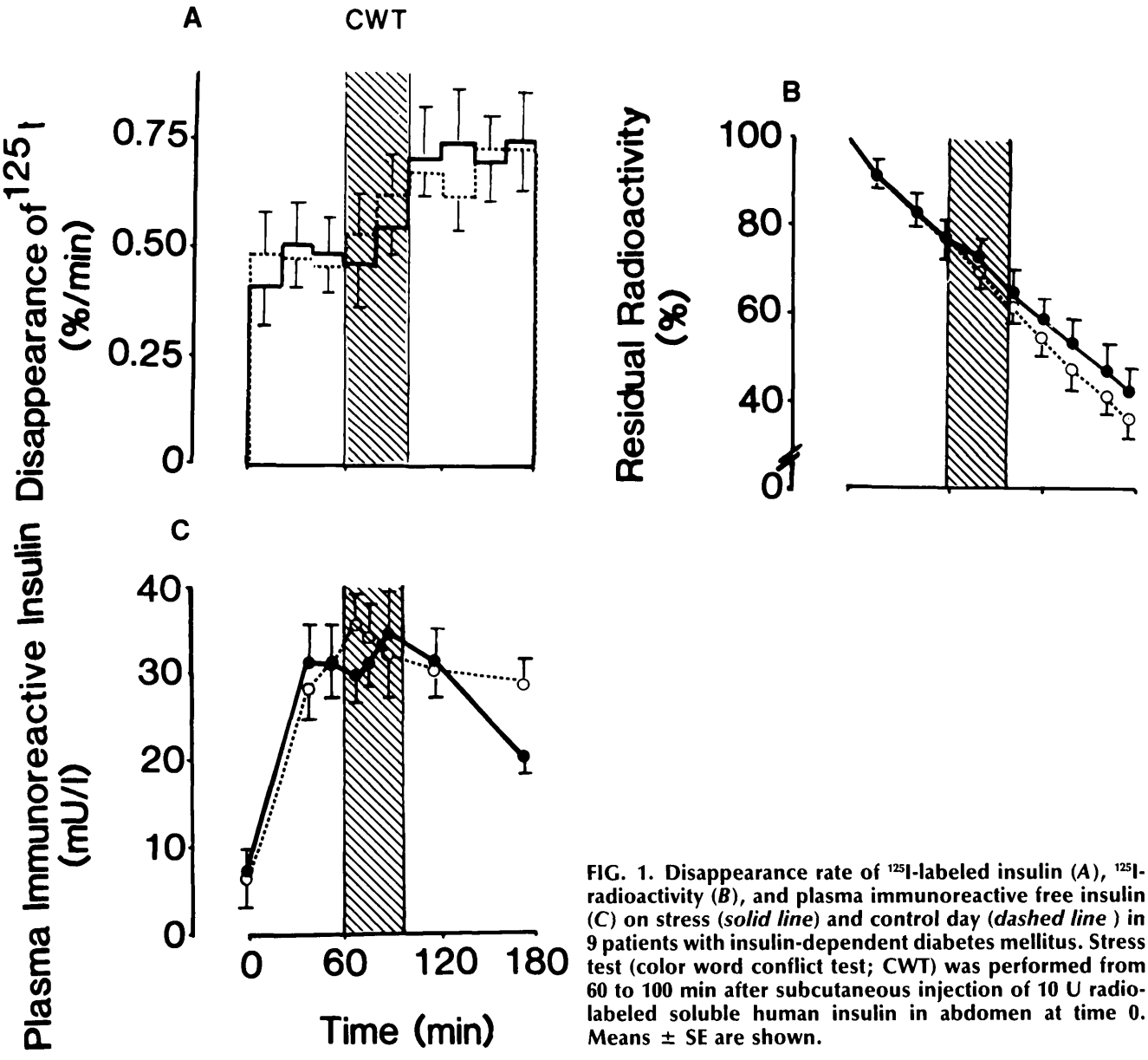

FIG. 1. Disappearance rate of ^{125}I-labeled insulin (*A*), ^{125}I-radioactivity (*B*), and plasma immunoreactive free insulin (*C*) on stress (*solid line*) and control day (*dashed line*) in 9 patients with insulin-dependent diabetes mellitus. Stress test (color word conflict test; CWT) was performed from 60 to 100 min after subcutaneous injection of 10 U radiolabeled soluble human insulin in abdomen at time 0. Means ± SE are shown.

measuring 0.89 ± 0.19 and 2.39 ± 0.62 nM, respectively ($P < 0.001$ and $P < 0.01$ vs. control day). Arterial plasma epinephrine and norepinephrine concentrations tended to level off during the stress test but remained significantly elevated throughout this and had normalized 20 min later. During the control day, both epinephrine and norepinephrine increased slightly over the study. This increase was significant for plasma norepinephrine, which measured 1.42 ± 0.16 nM ($P < 0.05$) at the end of the control study.

Basal abdominal adipose tissue blood flow did not differ significantly between stress and control days measuring 4.9 ± 0.8 and 4.5 ± 0.5 ml · min^{-1} · 100 g^{-1}, respectively (Fig. 2C). During the control day, adipose tissue blood flow decreased significantly from the first to the last 20-min period of the investigation ($P < 0.05$). During the first 20-min interval of the CWT, adipose tissue blood flow increased 83 ± 30% ($P < 0.05$), after which it returned to the basal level. Like the glycerol response to stress, adipose tissue blood flow showed a decrease followed by a second increase in the recovery period after the termination of the stress. Thus, after the CWT and onward, adipose tissue blood flow was again significantly higher than on the control day ($P < 0.05$). Adipose tissue vascular resistance decreased significantly during the first 20 min of the CWT compared with the control day (−16 ± 10 vs. 23 ± 8%, $P < 0.05$), after which no differences were found.

Basal heart rate and arterial blood pressure were similar on the test and control days measuring 64 ± 3 vs. 63 ± 4 beats/min and 129 ± 4/64 ± 2 vs. 131 ± 3/67 ± 3 mmHg. Maximal heart rate increases

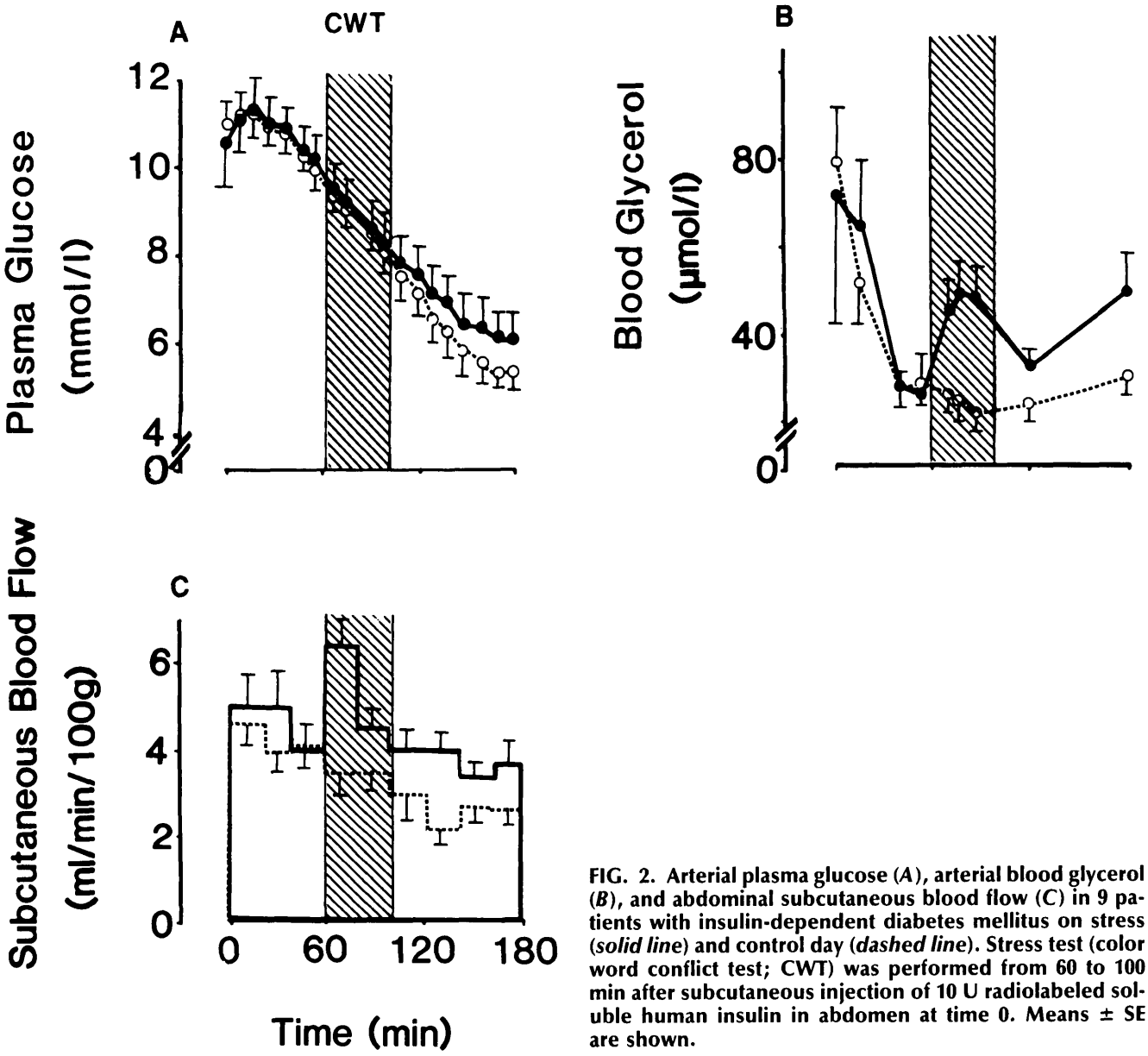

FIG. 2. Arterial plasma glucose (*A*), arterial blood glycerol (*B*), and abdominal subcutaneous blood flow (*C*) in 9 patients with insulin-dependent diabetes mellitus on stress (*solid line*) and control day (*dashed line*). Stress test (color word conflict test; CWT) was performed from 60 to 100 min after subcutaneous injection of 10 U radiolabeled soluble human insulin in abdomen at time 0. Means ± SE are shown.

of 36 ± 5 beats/min (P <0.001 vs. both control and basal levels) were found at 3 min of CWT, followed by a slight decline, but heart rate was still markedly elevated above basal values and the control day even during the last min of the CWT, measuring 89 ± 5 beats/min at 37 min of stress (P < 0.001). A similar response pattern was found for arterial blood pressure. Maximal systolic, diastolic, and mean blood pressure increases measured 29 ± 6, 22 ± 3, and 24 ± 4 mmHg, respectively (P < 0.001 vs. basal and control). Arterial blood pressure was normalized 20 min after the CWT.

CONCLUSIONS

This is the first study in patients with IDDM in which the absorption and bioavailability of the subcutaneously injected insulin have been investigated in connection with mental stress. The study shows, in accordance with our previous study in healthy subjects (27), that insulin absorption is also unaltered in IDDM patients during psychological stress. Furthermore, metabolic, sympathoadrenal, and hemodynamic responses to standardized mental stress were evaluated. It is shown that neither glucose control nor ketone body levels changed during this type of short-term mental stress. In contrast, the stress resulted in a significant elevation of the plasma catecholamine levels and of the lipolytic activity, with a doubling of blood glycerol levels both in the acute phase of the stress and in the recovery period. Concomitantly with the increased lipolysis, the blood flow in the subcutaneous tissue increased considerably. In fact, none of the responses in this study differed from those previously observed by us during CWT in healthy sub-

jects (27). Thus, both the sympathoadrenal reactivity and the lipolytic and hemodynamic responses were normal in this group of IDDM patients with normal autonomic nervous function. In contrast, according to one report, patients with severly impaired autonomic nervous function were unable to respond normally to psychological stress (28).

It is frequently believed that mental stress impairs glycemic control in diabetic subjects. However, our study does not support this assumption. In accordance with a well-controlled study, in which an arithmetic test and public speaking were used as stressors (5), no significant changes in blood glucose levels were evoked by the stress. Exposure to difficult life experiences (4), stressful interviews (1), and hypnosis (2) have been reported to result in alterations in glucose control. However, those studies are difficult to evaluate due to unsatisfactory definition of the stresses applied, small patient materials, and heterogeneous study groups. Furthermore, unlike our study and the one by Kemmer et al. (5), measurements were not provided of hormonal and cardiovascular variables with which to evaluate the stress intensity.

In contrast to the unaltered insulin absorption and glucose homeostasis during the CWT, lipolysis increased significantly during and after the stress, as shown by the elevated blood glycerol levels. Thus, the stress was accompanied by increased lipolysis to approximately the same degree in IDDM patients as previously found in healthy subjects (11,27). On the basis of free-fatty acid measurements, Kemmer et al. (5) in their well-controlled study suggested that psychological stress does not affect the lipolytic rate, either in healthy subjects or in patients with IDDM. However, data on free fatty acids are inferior to glycerol determinations for the evaluation of lipolysis, because the former can be reesterified in adipose tissue.

The increased lipolysis did not result in increased β-hydroxybuturate levels. Nor did the ketone body levels change during psychological stress in the study by Kemmer et al. (5). Thus, the two well-controlled studies on metabolic effects of mental stress in IDDM demonstrate unequivocally that short-term mental stress does not result in ketosis or disturbed glucose control in diabetic patients. However, it cannot be excluded that metabolic decompensation might result from stress of longer duration than the 40 min applied in this study. Also, the type of stress might be of importance. Many life stresses consist of prolonged worry rather than acute emotional arousal. Even if the direct neurogenic and hormonal effects of the stress do not result in metabolic disturbances, indirect effects such as altered dietary and therapy compliance might perhaps play a role during emotional stress of longer duration.

The subcutaneous blood flow was considerably increased in this study during and after the CWT, with a parallel decrease of the resistance in the adipose tissue. This is similar to the response previously reported by us in healthy subjects (11,27), a response considered to be caused by the concerted action of withdrawal of vasoconstrictor nerve activity, vascular β_2-adrenoceptor stimulation by circulating epinephrine, and increased lipolysis. Others have been unable to demonstrate an elevation of adipose tissue blood flow in patients with IDDM similar to that in healthy subjects during mental stress (29). The discrepancy between this and the latter study might depend on differences in patient materials. In the latter study, indications for parasympathetic nervous dysfunction were found in the IDDM patients, whereas our patients showed no evidence of autonomic neuropathy. Furthermore, the mental stress procedure applied in the former study was much milder.

Although adipose tissue blood flow was almost doubled during the CWT, insulin absorption was unaltered, as shown by the almost congruent curves of the disappearance rates over time on the test and control days. On both days, there was a continuous rise in the rate of disappearance during the measurement period, probably due to dissociation of the injected hexameric insulin into dimers and monomers (30). A potentiating effect on the dissociation of insulin by an increase in blood flow could be argued to be greater if the stress provocation had been carried out soon after injection when more hexameric insulin remained at the injection site. Thus, we might have overlooked an effect of the stress on insulin absorption by using a 1-h latent period between the injection and the stress provocation. However, this seems unlikely, because recent studies comparing the absorption of soluble insulin with that of monomeric insulin analogues suggest that a substantial amount of soluble insulin is still present as undissociated slowly absorbable complexes 1 h after the injection (30).

Consistent with the reported response during mental stress, insulin absorption is unaltered in response to an elevated circulating norepinephrine level, despite increased adipose tissue blood flow (E.F.-F., R. Gunnarson, B.L., unpublished observations). There are similarities between these two provocations, which both lead to blood pressure elevation concomitantly with the vasodilation. The blood pressure elevation might, via myogenic mechanisms (31), counteract the recruitment of capillaries normally occurring during vasodilatation, resulting in a lower rate of insulin absorption.

In summary, this study suggests that neither the absorption of subcutaneously injected soluble insulin nor glucose homeostasis or blood ketone body levels were significantly altered in patients with IDDM during 40 min of intense mental stress. In contrast, the lipolytic activity increased considerably, with a concomitant elevation of the adipose tissue blood flow during and after the stress.

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