Metabolic Studies in Chlorpropamide-Alcohol Flush Positive and Negative Type 2 (Non-Insulin Dependent) Diabetic Patients With and Without Retinopathy

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Summary. Serum insulin and blood metabolite responses to oral glucose with and without intravenous naloxone were measured in 24 chlorpropamide-alcohol flush positive and negative Type 2 (non-insulin dependent) diabetic patients with and without retinopathy. In the chlorpropamide-alcohol flush positive patients with retinopathy, fasting blood glucose was increased $> 40\%$ and the serum triglycerides were increased over twofold compared with each of the other three groups. Following oral glucose (50 g), the chlorpropamide-alcohol flush positive diabetic patients with complications had a lower serum insulin and higher blood glycerol than the other

three groups. Thus, chlorpropamide-alcohol flush positive subjects with retinopathy showed distinct metabolic differences from the other three groups. There was no evidence that opiate-receptors influenced the metabolic response to oral glucose in the Type 2 diabetic patients since the infusion of intravenous naloxone produced no effect on the serum insulin or blood metabolites.

Key words: Chlorpropamide-alcohol flush, retinopathy, glucose tolerance test, serum insulin, serum triglycerides, blood metabolites, Type 2 diabetes.

The facial flush which occurs in some Type 2 diabetic patients after drinking alcohol when taking chlorpropamide (chlorpropamide-alcohol flushing, CPAF) we have found to be associated with a decreased frequency of retinopathy, proteinuria and large vessel disease [1–3]. In view of this association and the possible contribution of metabolic factors in the development of these complications, we have studied the metabolic response to oral glucose in CPAF-positive and negative Type 2 diabetic subjects with and without severe retinopathy. Since CPAF is mediated, at least in some subjects, through opiate receptors [4], we have examined the effect of the opiate antagonist naloxone on the response in these groups to oral glucose.

Patients and Methods

Patients

Four groups of six Type 2 diabetic patients were studied (four males and two females in each group): (1) CPAF-positive without complications; (2) CPAF-positive with complications; (3) CPAF-negative without complications; (4) CPAF-negative with complications.

CPAF status had been determined during the previous 3–4 months by both subjective response and facial temperature measurement as described previously [4]. The groups were matched for age: group 1: 60 ± 6 years, group 2: 61 ± 5 years, group 3: $65 \pm$

3 years, group 4: 61 ± 5 years; duration of diabetes: group 1: 17 ± 1 years, group 2: 16 ± 3 years, group 3: 13 ± 1 years, group 4: 12 ± 1 years and percentage average body weight: group 1: $101 \pm 7\%$, group 2: $101 \pm 5\%$, group 3: $99 \pm 6\%$, group 4: $104 \pm 3\%$ (calculated from Metropolitan Life Assurance Tables, 1959). Those with complications had either maculopathy or proliferative retinopathy. Two subjects in group 1 and three in group 3 were on diet alone, the rest were on diet plus oral hypoglycaemic therapy (all but two on glibenclamide). Patients stopped their oral hypoglycaemic agents 2 weeks before the study and took no other medication; all gave consent to the investigation.

Procedure

On two separate occasions after an overnight fast an IV cannula was inserted into an antecubital vein and kept patent with 0.15 mol/l saline. After 30 min rest, blood was taken at 30 and 15 min and immediately before the oral administration of 50 g of glucose and then at 15 min intervals for 1 h and 30 min intervals for a further 2 h. On one occasion the patients received a bolus injection of 5 mg IV naloxone 15 min before the glucose, followed by an IV infusion of 0.053 mg/min of naloxone in 0.15 mol/l saline for 75 min. On the other occasion, an identical volume of 0.15 mol/l saline alone was given. The two studies were carried out single blind in random order at least 1 week apart.

Assays

Serum insulin was measured by radioimmunoassay [5] and blood lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate by a semi-

Table 1. Fasting blood glucose, serum insulin, serum triglycerides and blood metabolites in CPAF-positive and negative Type 2 diabetic patients with and without complications

Fasting levels	CPAF-positive patients		CPAF-negative patients	
	Without complications (group 1)	With complications (group 2)	Without complications (group 3)	With complications (group 4)
Glucose (mmol/l)	10.0 ± 1.4	14.3 ± 1.1*	10.1 ± 0.6	9.9 ± 1.5
Insulin (mU/l)	9.2 ± 3.2	6.2 ± 0.6	10.0 ± 2.9	8.5 ± 2.0
Triglycerides (mmol/l)	1.4 ± 0.3	3.9 ± 1.3*	1.3 ± 0.2	1.8 ± 0.5
Lactate (mmol/l)	0.90 ± 0.14	0.98 ± 0.09	0.98 ± 0.04	0.93 ± 0.07
Alanine (mmol/l)	0.29 ± 0.03	0.32 ± 0.03	0.32 ± 0.01	0.33 ± 0.01
Glycerol (mmol/l)	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.02
3-Hydroxybutyrate (mmol/l)	0.14 ± 0.03	0.29 ± 0.06	0.16 ± 0.05	0.12 ± 0.05
Pyruvate (mmol/l)	0.07 ± 0.00	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.00

Results expressed as mean ± SEM; * $p < 0.05$ compared with the other three groups

automated enzymatic fluorimetric technique [6]. Serum cholesterol and triglycerides were measured by standard automated enzymatic techniques.

Statistical Analysis

Results are expressed as mean ± SEM. Comparisons were made using the Wilcoxon 'U' test for non-parametric data.

Results

Fasting Values

The mean of the -30, -15 and 0 fasting blood glucose was significantly higher in the CPAF-positive group with complications than in the other groups, group 1: 14.3 ± 1.1 versus 10.0 ± 1.4 mmol/l; group 3: 10.1 ± 0.6 and group 4: 9.9 ± 1.5 mmol/l (Table 1). Serum triglycerides were also significantly increased in the same groups (3.9 ± 1.3 versus 1.4 ± 0.3 , 1.3 ± 0.2 , 1.8 ± 0.5 mmol/l, respectively). Serum insulin and blood lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate concentrations were similar in all four groups (Table 1).

Responses to an Oral Glucose Tolerance Test

The change in the blood glucose following oral glucose was similar in all four groups (mean difference from fasting values were group 1: 3.5 ± 0.8 , group 2: 4.0 ± 0.5 , group 3: 3.5 ± 0.7 and group 4: 4.0 ± 0.4 mmol/l, respectively). The mean change in the serum insulin, however, was less in the CPAF-positive group with complications than in the CPAF-positive group without complications (2.3 ± 1.3 and 3.2 ± 1.3 mU/l versus 9.6 ± 3.5 and 8.3 ± 0.7 mU/l at 60 and 150 min, respectively; $p < 0.05$ – significance not attained at other time points although mean delta insulin was always less in the group with complications). Blood glycerol was higher in the CPAF-positive group with complications than in the other three groups with significant differences compared with group 1 at 45, 120 and 150 min: group 2: 0.06 ± 0.01 versus 0.04 ± 0.01 mmol/l, group 3: $0.06 \pm$

0.01 versus 0.04 ± 0.01 mmol/l and group 4: 0.09 ± 0.01 versus 0.04 ± 0.01 mmol/l, and with group 4 at 120 and 180 min after the glucose load: 0.04 ± 0.01 versus 0.06 ± 0.01 and 0.05 ± 0.01 versus 0.10 ± 0.02 mmol/l, respectively). The blood pyruvate in the CPAF-positive patients with complications was higher than CPAF-positive patients without complications at 30 and 150 min (0.09 ± 0.01 versus 0.06 ± 0.01 and 0.09 ± 0.01 versus 0.06 ± 0.01 mmol/l, respectively). There were no consistent significant differences between the groups in the response of blood lactate, alanine, or 3-hydroxybutyrate (Table 1).

Responses to Naloxone

The infusion of IV naloxone produced no consistent changes in the response of serum insulin or blood glucose, lactate, alanine, pyruvate, glycerol or 3-hydroxybutyrate to oral glucose. The changes in blood glucose following oral glucose plus naloxone infusion (group 1: 4.0 ± 0.5 ; group 2: 4.2 ± 0.3 ; group 3: 3.8 ± 0.5 and group 4: 3.1 ± 0.9 mmol/l, respectively) were not significantly different from those seen without naloxone. Naloxone also had no significant effect on the change in serum insulin after the glucose load (mean change in serum insulin without naloxone was group 1: 8.7 ± 0.3 ; group 2: 3.9 ± 1.2 ; group 3: 5.6 ± 4.2 and group 4: 9.2 ± 3.4 mU/l and with naloxone group 1: 7.5 ± 6.2 ; group 2: 2.0 ± 0.8 ; group 3: 11.4 ± 5.7 and group 4: 7.1 ± 2.2 mU/l respectively).

Discussion

CPAF-positive Type 2 diabetic patients with retinopathy showed metabolic differences from the other three groups of patients. They had increased fasting blood glucose and serum triglycerides and following oral glucose a decreased level of serum insulin and increased blood glycerol concentration.

It has previously been suggested that Type 2 diabetic subjects with retinopathy have a lower serum insulin

response to a glucose load than those without [7, 8]. In one of these studies, patients with retinopathy had fasting serum cholesterol and triglyceride levels which correlated with the impairment of the insulin response [7]. Our results are in agreement with these observations in the CPAF-positive group. In the CPAF-negative Type 2 diabetic patients fasting serum triglycerides and insulin responses to oral glucose were the same in those with and without retinopathy. The present study does not determine whether this is due to differences in the aetiology of diabetic retinopathy in the two groups or merely that the CPAF group of patients, who seem to be partially protected from retinopathy, must be metabolically worse than their CPAF-negative counterparts before they develop complications.

Both stimulation and inhibition of insulin secretion have been demonstrated in vitro in response to an enkephalin analogue, the response varying according to dose [9]. However, neither naloxone nor an enkephalin analogue in vivo produced an effect on serum insulin [10, 11]. In the present study, naloxone did not influence serum insulin or blood metabolite response to oral glucose. This observation therefore does not support the idea that increased sensitivity to endorphins may be involved in the pathogenesis of certain forms of Type 2 diabetes [4].

This study suggests that CPAF-positive patients with retinopathy may form a metabolically distinct group of non-insulin dependent diabetic patients. Whether these observations are relevant to the aetiology of retinopathy in Type 2 diabetes remains undetermined.

Acknowledgements. We are grateful to the British Diabetic Association for financial support. AHB was supported by the Medical Research Council.

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Received: 16 July 1982

and in revised form: 31 August 1982

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