

The Effect of Treatment of Type 2 (Insulin Independent) Diabetes Mellitus on Plasma Concentrations of Pancreatic Polypeptide and Glucagon

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Summary. The effect of the control of diabetes with diet and insulin upon plasma levels of human pancreatic polypeptide and glucagon was determined in eight patients with Type 2 (insulin independent) diabetes mellitus. The mean \pm SEM fasting plasma glucose was 15.9 ± 1.3 mmol/l for 5 days of diet treatment and 5.9 ± 0.4 mmol/l for the last 5 days of treatment with diet plus insulin ($p < 0.0001$); corresponding fasting plasma pancreatic polypeptide levels were 328 ± 97 and 247 ± 71 pg/ml ($p < 0.05$) and immunoreactive glucagon levels were 95 ± 11 and 62 ± 6 pg/ml ($p < 0.005$). Cooked ground beef was administered on the first day of diet treatment and on the last day of treatment with diet plus insulin; mean maximal rise of pancreatic polypeptide, and total and incremental plasma pancreatic polypeptide response areas were significantly lower following treatment ($p < 0.01$), as was total area for immunoreactive glucagon ($p < 0.05$). Normalisation of fasting plasma glucose by short-term treatment with diet plus insulin is associated with decreases in basal and stimulated secretory activity of the pancreatic polypeptide cells in insulin independent diabetes mellitus.

Key words: Insulin independent diabetes mellitus, human pancreatic polypeptide, glucagon, control of diabetes, insulin treatment, protein meal.

The ingestion of a protein meal is a potent stimulus for the rapid release of immunoreactive human pancreatic polypeptide (PP) in healthy subjects [1–3]. In a group of patients with Type 2 diabetes mellitus (insulin independent) under treatment with diet plus insulin mean basal plasma level of human PP was

elevated [2]. The current investigation was carried out to determine whether short-term control of Type 2 diabetes with diet plus insulin, sufficient to bring fasting plasma levels of glucose into the normal range, would have an effect upon basal and beef meal-stimulated plasma levels of human PP.

Materials and Methods

Patients

Eight patients with uncontrolled diabetes mellitus were studied in the Clinical Research Center of University of Michigan Hospital after giving their informed consent. The investigations were performed in accordance with the principles of the Declaration of Helsinki. The clinical data are shown in Table 1. The patients were classified as having Type 2 diabetes because: 1) after a mean known duration of diabetes of 8 years (range 0–16 years), none had had ketonuria or ketoacidosis (only three patients had ever received insulin, each for less than 2 weeks), 2) the age at diagnosis ranged from 26 to 59 years, 3) at this study each had a fasting plasma insulin level in the normal range and an increase after ingestion of beef (mean maximal increase was 17 mU/l).

No patient had evidence of circulating antibodies to PP [2] as ascertained by incubation of plasma with ^{125}I -PP followed by precipitation of plasma proteins with polyethylene glycol [2]. Patients 1, 2, and 5 had received treatment with insulin, and patients 3 and 7 sulphonylureas; therapy had been discontinued 1 week before hospitalisation. At the time of admission six patients had mild to moderate polyuria and polydipsia. Patients 1 and 3 had necrobiosis lipoidica diabetorum. Except for diabetes, only one patient had a major illness; patient 6 had mild hypertension, treatment for which had been discontinued 10 days before admission. No patient received any medication other than insulin during the study.

Treatment and Testing of Patients

Upon admission the patients were placed on diets which were calculated to maintain their current weight throughout the hospitalisation; the caloric value of each patient's diet was derived 40% from carbohydrate, 40% from fat and 20% from protein. Following an initial 5 days of study on diet alone (period 1), insulin

Table 1. Clinical data of patients with Type 2 (insulin independent) diabetes mellitus

Patient	Sex	Age (years)	Ideal body weight ^a (%)	Known duration of diabetes (years)	Diet (kcal/day)	Average insulin dose (U/day) ^b	Interval between first and second beef meal (days)
1	F	26	95	0	1,540	23	16
2	M	33	86	4	2,150	30	17
3	M	34	120	4	2,155	53	16
4	M	44	127	6	1,800	17	14
5	F	51	131	11	1,800	55	16
6	M	63	94	16	2,100	34	20
7	F	67	105	15	1,835	35	17
8	F	68	98	9	1,600	34	14

^a Based on Metropolitan Life Insurance Company tables derived primarily from data of the Build and Blood Pressure Study, Society of Actuaries, 1959

^b Dose during the 5 days of optimal control (period 2)

therapy was begun. Patients received doses of lente and soluble insulin 30 min before breakfast and again at 1400 h daily. Each patient's average daily dose of insulin is shown in Table 1. When a normal fasting plasma glucose was attained (after 4–10 days of treatment), 5 days of observations were made while optimal insulin therapy was continued (period 2). As an additional index of glycaemic control 24 h urinary excretion of glucose was measured daily.

A test meal of 125 g cooked ground beef was ingested on the second hospital day (on the first day of period 1) following the overnight fast. A butterfly needle was inserted into an antecubital vein and maintained patent by a solution of heparin (100 U/ml) in 0.154 mol/l saline. Samples of venous blood were obtained and placed into heparinised tubes 30, 15 and 0 min before and 5, 10, 20, 30, 60, 120 and 180 min after the start of the ingestion. The test was repeated on the last day of insulin treatment (period 2) when it was begun 60 min after the morning dose of insulin. All patients consumed the beef meal over 5–10 min periods. On the morning of beef ingestion, the –15 and 0 min samples were considered that day's fasting (basal) samples and the mean value of these two samples was considered the fasting level. On the other eight mornings of observation, a blood sample was obtained after the overnight fast and before the morning dose of insulin. All samples were placed in ice immediately; the plasma was separated within 2 h and stored at –20 °C until assayed.

Assays

Plasma PP [4], immunoreactive glucagon (IRG) [5] and immunoreactive insulin (IRI) [6] were measured by double-antibody radioimmunoassays. Standard human PP (lot 615-10543-200) and rabbit anti-human PP serum (lot 615-1054B-248-18) were given to us by Dr. Ronald Chance of the Eli Lilly, Indianapolis, Indiana, USA. The glucagon antiserum reacts with the carboxyl-terminal region of glucagon; it reacts negligibly with glucagon-like immunoreactivity of jejunal extracts [5]. Plasma glucose was measured by a hexokinase method using a Gilford 3500 computer-directed spectrophotometer [7]. Urinary glucose was measured by a glucose oxidase method [8] using a Beckman System I Analyser. Radioimmunoassay results were computed using a computer program (RIANAL) [9]. All plasma samples from a patient were analysed in a single PP assay and a single glucagon assay; four assays of each peptide were performed. All data are presented as mean \pm SEM. The effect of insulin treatment upon plasma levels of glucose, PP and IRG was ascertained by comparing the mean of each patient's fasting levels during period 1 with

that of period 2 and by comparing total and incremental PP and IRG areas in response to beef meals during periods 1 and 2. As there were eight patients, there were eight pairs of observations for basal levels and for each response area. The statistical comparisons were made by two-tailed t-tests for paired or unpaired observations [10].

Results

Fasting Levels

Fasting plasma levels of glucose, PP and IRG for each patient and for the group are shown in Table 2. The fasting glucose level of the group was 15.9 ± 1.3 mmol/l in period 1 and 5.9 ± 0.4 mmol/l in period 2 ($p < 0.0001$); during period 2 the mean levels of individuals ranged from 4.9 to 6.8 mmol/l. Mean \pm SEM 24 h urinary glucose was 47 ± 19 g in period 1 and 2 ± 1 g in period 2 ($p < 0.05$).

Fasting plasma PP decreased from 328 ± 97 pg/ml in period 1 to 247 ± 71 pg/ml during period 2 ($p < 0.05$). Mean fasting PP was lower in five patients, increased in one and remained unchanged in two. Fasting IRG level in period 1 was 95 ± 11 pg/ml and in period 2 was 62 ± 6 pg/ml ($p < 0.005$); fasting IRG levels were lower in period 2 than in period 1 in all patients.

Fasting plasma levels of glucose, PP and IRG for each patient on days 1 and 5 (the last day) of period 1 are shown in Table 3; there was no significant difference in the mean levels of glucose, PP or IRG on day 1 compared with day 5.

Responses to Beef Meal

During the period 1 beef test plasma glucose declined gradually from the baseline of 16.6 ± 0.8 mmol/l to a nadir of 15.0 ± 1.1 mmol/l at 180 min ($p < 0.05$)

Table 2. Effect of treatment of Type 2 (insulin independent) diabetes on fasting plasma glucose, pancreatic polypeptide (PP) and immunoreactive glucagon (IRG). Mean \pm SEM fasting plasma levels of glucose, PP and IRG for 5 days of dietary treatment (period 1) and for 5 consecutive days during treatment with diet plus insulin immediately after fasting plasma glucose had been brought into or near the normal range (period 2)

Patient	Dietary treatment (period 1)			Treatment with diet and insulin (period 2)		
	Glucose (mmol/l)	PP (pg/ml)	IRG (pg/ml)	Glucose (mmol/l)	PP (pg/ml)	IRG (pg/ml)
1	15.4 \pm 0.4	143 \pm 18	116 \pm 6	5.9 \pm 0.4	72 \pm 4	71 \pm 8
2	13.6 \pm 0.2	18 \pm 4	157 \pm 16	4.9 \pm 0.3	40 \pm 7	77 \pm 12
3	16.4 \pm 0.4	216 \pm 27	108 \pm 18	5.7 \pm 0.2	198 \pm 28	62 \pm 6
4	9.3 \pm 0.7	32 \pm 10	91 \pm 14	6.1 \pm 0.4	37 \pm 7	82 \pm 8
5	21.9 \pm 1.1	569 \pm 89	89 \pm 12	6.3 \pm 0.6	423 \pm 47	52 \pm 10
6	18.6 \pm 0.3	365 \pm 35	65 \pm 8	6.0 \pm 0.2	368 \pm 23	45 \pm 12
7	17.4 \pm 0.06	789 \pm 34	54 \pm 7	6.2 \pm 0.6	583 \pm 57	34 \pm 5
8	14.6 \pm 0.4	491 \pm 78	84 \pm 12	6.8 \pm 0.4	256 \pm 30	75 \pm 10
Mean	15.9	328	95	5.9	247	62
\pm SEM	1.3	97	11	0.2	71	6
p-value	<0.0001			<0.005		

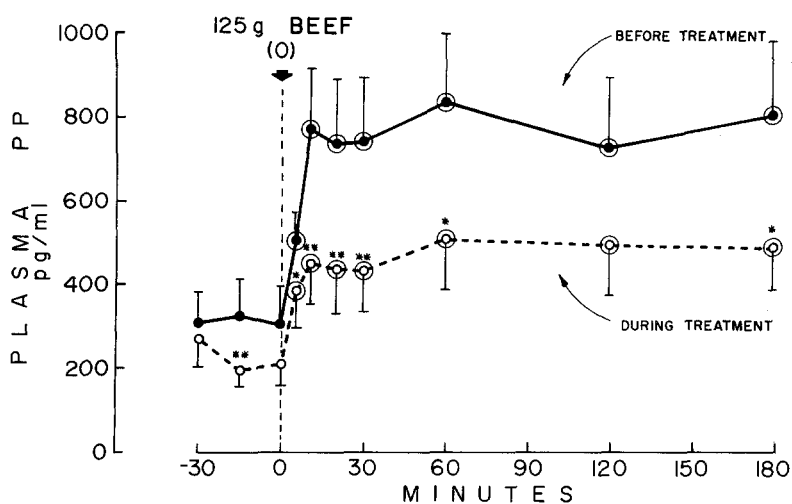


Fig. 1. Effect of the ingestion of test meals of 125 g cooked ground beef on mean \pm SEM plasma pancreatic polypeptide (PP) levels during treatment with diet (period 1) and during treatment with diet plus insulin (period 2) in eight patients with Type 2 diabetes. Test meals ingested on the first (mean \pm SEM fasting plasma glucose, 16.6 \pm 0.8 mmol/l) of the 5 days before insulin treatment and on the last day (mean \pm SEM fasting plasma glucose, 6.3 \pm 0.5 mmol/l) of treatment with diet plus insulin. Circles around the individual points denote significant differences from basal levels ($p < 0.05$). Asterisks denote significant differences after treatment of diabetes. * $p < 0.05$, ** $p < 0.02$

Table 3. Plasma levels of glucose, pancreatic polypeptide and immunoreactive glucagon in Type 2 diabetes on days 1 and 5 of period 1. Mean \pm SEM fasting plasma levels of glucose, PP, and IRG for days 1 and 5 of dietary treatment (period 1)

Patient	Glucose (mmol/l)		Pancreatic polypeptide (pg/ml)		Immunoreactive glucagon (pg/ml)	
	day 1	day 5	day 1	day 5	day 1	day 5
1	16.0	16.2	137	117	137	112
2	13.5	13.1	8	27	165	111
3	15.7	17.4	258	267	120	81
4	11.3	8.7	47	15	122	98
5	17.8	23.5	375	904	79	134
6	18.6	17.8	365	300	65	39
7	17.6	17.2	715	760	52	37
8	13.3	14.7	397	486	94	107
Mean	15.4	16.1	288	360	104	90
\pm SEM	0.9	1.5	81	118	14	13
p-value	NS		NS		NS	

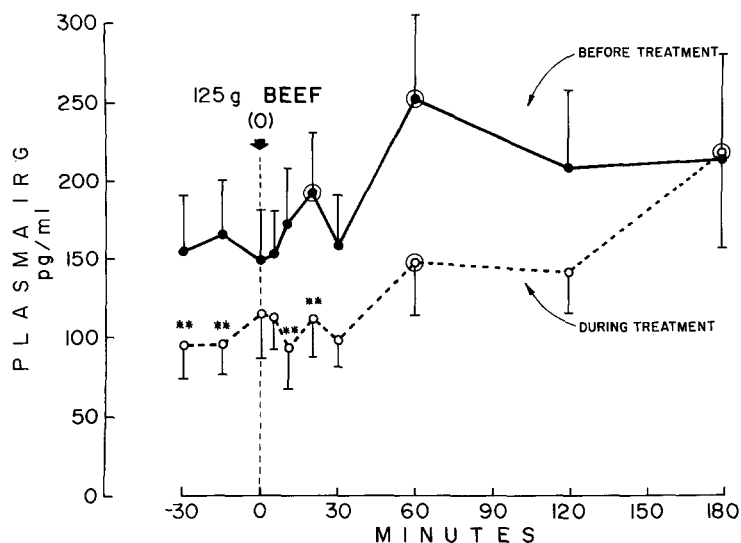


Fig. 2. Effect of the ingestion of test meals of 125 g cooked ground beef on mean \pm SEM plasma immunoreactive glucagon (IRG) levels during periods 1 and 2. See also legend for Fig. 1

Table 4. Effect of treatment of Type 2 diabetes on response of plasma pancreatic polypeptide and immunoreactive glucagon to ingestion of beef. Plasma PP and IRG response areas following ingestion of 125 g cooked ground beef during dietary treatment (period 1) and during optimal treatment with insulin plus diet (period 2) in eight patients

	Pancreatic polypeptide (ng. min ⁻¹ ml ⁻¹)		Immunoreactive glucagon (ng. min ⁻¹ ml ⁻¹)	
	Total area	Incremental area	Total area	Incremental area
Period 1	137 \pm 26	81 \pm 9	38 \pm 9	9 \pm 5
Period 2	86 \pm 18	50 \pm 15	26 \pm 15	7 \pm 3
p-value	< 0.01	< 0.005	< 0.05	NS

Results are expressed as areas above zero plasma levels and designated total area and as areas above the basal level at the start of the ingestion of beef (average of -15 and 0 min plasma levels) and designated incremental area

(levels not shown). During the beef test of period 2 plasma glucose decreased from 6.3 ± 0.5 to 4.9 ± 0.5 mmol/l at 180 min ($p < 0.05$) and was significantly lower at all time points than during the period 1 test ($p < 0.0001$). Plasma IRI values (mU/l) of the period 1 and period 2 beef tests respectively, were: at 0 min 8 ± 2 versus 21 ± 2 ; 30 min 12 ± 2 versus 26 ± 3 ; 60 min 22 ± 5 versus 31 ± 4 ; 120 min 23 ± 4 versus 35 ± 3 ; 180 min 19 ± 3 versus 32 ± 2 .

Mean plasma PP levels for periods 1 and 2 beef tests are shown in Fig. 1. During the period 2 test, mean plasma levels of PP at -15, 5-60 and 180 min and the mean maximal rise (746 ± 144 versus 474 ± 99 pg/ml, $p < 0.005$) were significantly lower than during the period 1 test. During the period 2 test the mean total PP area and the mean incremental PP area decreased significantly (Table 4); these areas decreased in seven of the eight patients.

During the period 1 beef ingestion plasma IRG (Fig. 2) levels rose significantly from a basal level of 157 ± 34 to 192 ± 39 pg/ml at 20 min; the peak of 253 ± 53 pg/ml was reached at 60 min. During the

period 2 test mean plasma IRG did not rise significantly until 60 min; the peak mean level occurred at 180 min. Mean levels of IRG were significantly lower than in the period 1 test at -30, -15, 10 and 20 min. The total IRG area decreased significantly during period 2 (Table 4).

Discussion

This investigation has shown that treatment of Type 2 (insulin independent) diabetes mellitus which brought mean fasting plasma glucose into the normal range was associated with a significant decrease in the mean plasma levels of PP. Before treatment with insulin, basal plasma levels of PP were supernormal in half of the patients, that is, $>$ mean + 2 SD of levels of healthy subjects in the same decades of age [4]; during treatment the fasting level of PP fell in five of the eight patients and the plasma PP response to the test meal was decreased in seven of the patients. The latter finding suggests that the effects of

the treatment include a decrease in the rate of secretion of PP. The mechanism by which this could happen was not the subject of study. It may relate to a change (improvement) in the metabolism of the PP cell resulting from the effects of insulin treatment. Such effects might include an improved recognition of glucose by the PP cell or an increase in metabolism of glucose by the PP cell. The diet treatment per se could have played a role in the lowering of mean basal and stimulated plasma levels of PP perhaps through a change in gastrointestinal function; there was, however, no difference in the mean fasting levels of PP or glucose of the first day as compared with day 5 of period 1 when diet was the only treatment. Improvement in impaired renal function [11, 12] would be expected to lower plasma levels of PP, but none of the patients had evidence of renal impairment.

During treatment there was a significant decrease in the mean fasting plasma level of IRG and in the mean total IRG response to the beef meal. The individual fasting levels of IRG which were normal [4] in period 1 decreased in each of the eight patients; the response to the meal decreased in five of the eight patients. Perhaps the much smaller plasma IRG than PP response to the meal made it more difficult to show consistently the effects of treatment upon the IRG response. Others found that the IRG response to protein meals was not lowered in Type 2 diabetes even when supraphysiological doses of insulin were infused overnight and supplemental injections of insulin were given shortly before the meals [13, 14]. The lowering of the IRG response to protein meals in the present study may be due to the longer preceding period of insulin treatment.

The treatment of patients with Type 2 diabetes resulted therefore in changes in plasma PP and IRG which qualitatively were similar. Treatment with diet plus insulin may have improved by the same mechanism abnormal metabolism of the PP cell and the A cell (secondary to the diabetic state) which appeared to have led to exaggerated hormonal responses to protein meals. Analogies concerning PP and A cell secretory functions must be made with circumspection. Differences in mechanisms of release of PP and IRG in healthy subjects are suggested for example by the differential effects of arginine infusion; infusion of arginine is without effect upon plasma levels of PP but induces marked increases in IRG [1, 2].

Among the patients of this study basal plasma levels of PP correlated with age ($r = 0.78$; $p = 0.02$) and when corrected for the effects of age the PP levels correlated with glucose levels of the period before insulin treatment was begun ($r = 0.69$; $p = 0.05$). The findings are in concert with earlier find-

ings that plasma levels of PP correlate with age in Type 2 diabetes [2] and in healthy subjects [2, 4, 15–17]. In three of the four older patients period 1 basal levels of PP were supernormal and in the fourth it was nearly so. Perhaps duration of diabetes or age interacting with diabetes relates to these findings in the older subjects. There was no evidence of an effect of adiposity upon fasting plasma levels of PP [18, 19].

The physiological significance of a reduction in fasting and post-prandial plasma levels of PP remains to be delineated. When human PP is infused in healthy subjects so as to raise PP to levels similar to those which occur post-prandially the rate with which trypsin appears in duodenal fluid is reduced [20]. The lowering of post-prandial levels of PP as achieved in these patients as a consequence of the control of diabetes could be expected to increase the rate with which trypsin appears in duodenal juice and to aid in the assimilation of protein.

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