

Intact Proinsulin and β -Cell Function in Lean and Obese Subjects With and Without Type 2 Diabetes

MICHAEL E. RØDER, MD
BO DINESEN, MSC
SVEND G. HARTLING, MD
PAULE HOUSSA, PHD

HENRIK VESTERGAARD, MD
FRANÇOISE SODOYEZ-GOFFAUX, PHD
CHRISTIAN BINDER, DMS

OBJECTIVE — Type 2 diabetes is a heterogeneous disease in which both β -cell dysfunction and insulin resistance are pathogenetic factors. Disproportionate hyperproinsulinemia (elevated proinsulin/insulin) is another abnormality in type 2 diabetes whose mechanism is unknown. Increased demand due to obesity and/or insulin resistance may result in secretion of immature β -cell granules with a higher content of intact proinsulin.

RESEARCH DESIGN AND METHODS — We investigated the impact of obesity on β -cell secretion in normal subjects and in type 2 diabetic patients by measuring intact proinsulin, total proinsulin immunoreactivity (PIM), intact insulin, and C-peptide (by radioimmunoassay) by specific enzyme-linked immunosorbent assays in the fasting state and during a 120-min glucagon (1 mg i.v.) stimulation test. Lean (BMI 23.5 ± 0.3 kg/m²) (LD) and obese (30.1 ± 0.4 kg/m²) (OD) type 2 diabetic patients matched for fasting glucose (10.2 ± 0.6 vs. 10.3 ± 0.4 mmol/l) were compared with age- and BMI-matched lean (22.4 ± 0.6 kg/m²) (LC) and obese (30.8 ± 0.9 kg/m²) (OC) normal control subjects.

RESULTS — Diabetic patients (LD vs. LC and OD vs. OC) had elevated fasting levels of intact proinsulin: 6.6 ± 1.0 vs. 1.6 ± 0.3 pmol/l and 7.7 ± 2.0 vs. 1.2 ± 0.2 pmol/l; PIM: 19.9 ± 2.5 vs. 5.4 ± 1.0 pmol/l and 29.6 ± 6.1 vs. 6.1 ± 0.9 pmol/l; and total PIM/intact insulin: 39 ± 4 vs. $15 \pm 2\%$ and 35 ± 5 vs. $13 \pm 2\%$, all $P < 0.01$. After glucagon stimulation, PIM levels were disproportionately elevated (PIM/intact insulin based on area under the curve analysis) in diabetic patients (LD vs. LC and OD vs. OC): 32.6 ± 6.7 vs. $9.2 \pm 1.1\%$ and 22.7 ± 5.2 vs. $9.1 \pm 1.1\%$, both $P < 0.05$. Intact insulin and C-peptide net responses were significantly reduced in type 2 diabetic patients, most pronounced in the lean group. The ratio of intact proinsulin to PIM was higher in diabetic patients after stimulation in both LD versus LC: 32 ± 3 vs. $23 \pm 2\%$, and OD versus OC: 28 ± 4 vs. $16 \pm 2\%$, both $P < 0.01$. In obese normal subjects, intact proinsulin/PIM was lower both in the fasting state and after glucagon stimulation: OC versus LC: 22 ± 3 vs. $33 \pm 3\%$ (fasting) and 16 ± 2 vs. $23 \pm 2\%$ (stimulated), both $P < 0.05$.

CONCLUSIONS — Increased secretory demand from obesity-associated insulin resistance cannot explain elevated intact proinsulin and disproportionate hyperproinsulinemia in type 2 diabetes. This abnormality may be an integrated part of pancreatic β -cell dysfunction in this disease.

Diabetes Care 22:609–614, 1999

From the Steno Diabetes Center (M.E.R., B.D., S.G.H., H.V., C.B.), Gentofte, Denmark; and the Centre Hospitalier Universitaire (P.H., F.S.-G.), Liège, Belgium.

Address correspondence and reprint requests to Michael E. Røder, MD, Rigshospitalet, Department of Endocrinology P, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. E-mail: mir@dadlnet.dk.

Received for publication 3 September 1998 and accepted in revised form 10 December 1998.

C.B. holds stock in and has served as a consultant to Novo Nordisk.

Abbreviations: AUC, area under the curve; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LC, lean control subjects; LD, lean type 2 diabetic patients; OC, obese control subjects; OD, obese type 2 diabetic control patients; PIM, total proinsulin immunoreactivity.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Type 2 diabetes is a heterogeneous disease in which both dysfunction of the pancreatic β -cells and decreased peripheral sensitivity to insulin are crucial to pathogenesis (1). Type 2 diabetic patients have impaired β -cell responses to both glucose and nonglucose secretagogues (2). The acute insulin response to intravenous glucose is nearly absent in type 2 diabetes already at mild hyperglycemia (6–7 mmol/l), whereas the acute insulin response to nonglucose secretagogues is impaired, but still measurable, at higher levels of hyperglycemia (2–4). Decreased insulin sensitivity in type 2 diabetes is probably linked to genetic components promoting insulin resistance and to a component of insulin resistance linked to obesity (2,5). Obesity is present in 50–80% of the individuals in various type 2 diabetes populations (5–7).

An elevated proinsulin-to-insulin ratio in the circulation is a well-known abnormality in type 2 diabetes (2,8–10). The exact mechanism for this increase is unknown. It has been hypothesized that an elevated proinsulin-to-insulin ratio is due to increased secretory demand on the β -cells because of insulin resistance and continuous hyperglycemia resulting in release of immature granules with a higher relative content of proinsulin and its conversion intermediates. This is not supported, however, by studies of experimental insulin resistance induced by nicotinic acid in baboons (11) or humans (12), or by observations in normal obese subjects (13), in which unchanged proinsulin-to-insulin ratios were found. We have recently shown that an elevated proinsulin-to-insulin ratio in type 2 diabetes is highly correlated with the degree of decreased insulin secretory capacity (14). However, it is not known how β -cell dysfunction in type 2 diabetes relates to conversion of intact proinsulin to insulin or whether a combination of obesity-associated insulin resistance has a differential effect on this conversion process. One reason has been lack of specific methods for measurement of the individual insulin precursors in the circulation (15–17).

In this study, we addressed whether absolute and relative levels of intact and

Table 1—Basic clinical characteristics of type 2 diabetic patients and control subjects

	Control subjects		Type 2 diabetic patients	
	Lean	Obese	Lean	Obese
<i>n</i>	10	9	11	10
Age (years)	51 ± 3	51 ± 2	57 ± 2	55 ± 2
Fasting β-glucose (mmol/l)	4.4 ± 0.1	4.8 ± 0.1	10.2 ± 0.6*	10.3 ± 1.1*
1-h β-glucose (mmol/l)	7.3 ± 0.3	7.3 ± 0.5	—	—
2-h β-glucose (mmol/l)	5.4 ± 0.3	5.5 ± 0.5	—	—
HbA _{1c} (%)	5.4 ± 0.1	5.4 ± 0.1	8.9 ± 0.4*	8.6 ± 0.5*
Duration of diabetes (years)	—	—	5.1 ± 1.4	5.5 ± 1.2
BMI (kg/m ²)	22.4 ± 0.6 (19.3–25.2)	30.8 ± 0.9 (27.3–37.3)†	23.5 ± 0.3 (22.5–25.9)	30.1 ± 0.4 (27.4–32.3)†

Data are means ± SEM or ranges. **P* < 0.01 for lean type 2 diabetic patients vs. lean control subjects or obese type 2 diabetic patients vs. obese control subjects; †*P* < 0.01 for obese vs. lean subjects (type 2 diabetic patients as well as control subjects).

total proinsulin levels are affected by obesity with or without concomitant β-cell dysfunction by using specific enzyme-linked immunosorbent assays (ELISAs) for intact proinsulin, total proinsulin immunoreactivity (PIM), intact insulin, and C-peptide. Lean and obese patients with type 2 diabetes and BMI-matched normal subjects were studied in the fasting state and under stimulated conditions.

RESEARCH DESIGN AND METHODS

Subjects

The study comprised 19 healthy subjects and 21 islet cell antibody-negative patients with type 2 diabetes who had a plasma C-peptide >600 pmol/l after intravenous glucagon stimulation (18). The patients consisted of a group of lean subjects (*n* = 11) and a group of obese subjects (*n* = 10) (Table 1) and the two groups of patients were matched according to fasting glycemia so that β-cell function could be compared (19). The normal subjects did not receive any medication, had no family history of diabetes, and were normotensive (blood pressure below 140/90 mmHg). They were BMI-matched to the group of lean type 2 diabetic patients (*n* = 10) and to the group of obese type 2 diabetic patients (*n* = 9). The clinical characteristics of the various groups are shown in Table 1. None of the subjects had any clinical or biochemical evidence of cardiac, hepatic, kidney, or thyroid function abnormalities. All participants were Caucasian.

Procedures

All subjects were studied in the morning after a 10-h overnight fast. An intravenous line was established in the antecubital vein,

and the subject rested in the supine position for at least 15 min before sampling. Blood samples were taken for measurement of intact proinsulin, PIM, insulin, and C-peptide and of basal clinical biochemical parameters. Samples for blood glucose measurements were taken from the earlobe. A 120-min glucagon test was then performed at the prevailing blood glucose, giving 1.0 mg of glucagon at time 0 intravenously over 30 s, followed by blood sampling after 6, 10, 20, 30, 60, 90, and 120 min for PIM, insulin, C-peptide, and intact proinsulin (only 0 and 6 min). All of the control subjects underwent a standard (75-g) oral glucose tolerance test on an additional day, ensuring that all had normal glucose tolerance (Table 1) according to World Health Organization criteria (20). The study was approved by the regional Ethical Committee, and informed oral and written consent was obtained from all participating subjects.

Analytical methods

Blood glucose was measured by a hexokinase method; basic clinical biochemical parameters, including creatinine, liver transaminases, alkaline phosphatase, thyrotropin, electrolytes, hemoglobin, total cholesterol, and triglycerides, were analyzed by routine laboratory methods. Islet cell antibodies were determined as previously described (21). HbA_{1c} was analyzed by high-performance liquid chromatography (HPLC) (Diamat7; Biorad, Hercules, CA); the reference range was 4.4–6.3%. PIM was analyzed by ELISA (22), using the monoclonal antibodies PEP-001 (anti-C-peptide) and HUI-001 (anti-insulin). The detection limit was 0.25 pmol/l; the interassay coefficient of variation (CV) was 8.7%; and molar cross-reactivities of proinsulin

conversion intermediates were 65–99% that of intact proinsulin. Intact proinsulin was measured by ELISA with the same methodological set-up as in Kjems et al. (22) except for a change in monoclonal antibodies. S2 (anti-AC-junction) was used as a coating antibody, and S53 (anti-BC-junction) as a biotin-labeled detection antibody. By using these two antibodies in a sandwich, an assay with 100% specificity against intact proinsulin was developed (23). All proinsulin conversion intermediates, insulin, and C-peptide cross-reacted <0.1% in this assay. For C-peptide, there was no interference below 50,000 pmol/l (23). The detection limit was 0.2 pmol/l of intact proinsulin, and interassay CV was 8.9%. Insulin was also measured by ELISA (24), without the cross-reactivity of intact proinsulin or split(32-33)- or des(31,32)-proinsulin. The detection limit in this assay was 5.0 pmol/l, and the assay had an interassay CV of 8.6%. C-peptide was determined by radioimmunoassay (25), using antibody M1230 (26). The detection limit was 60 pmol/l, and the interassay CV was 8.0%. Cross-reactivity with intact proinsulin was 13% and was 15% with des(31,32)-proinsulin (27).

Statistics and calculations

Student's *t* test was used for evaluation of differences between groups (two-tailed). All results are listed as means ± SEM unless otherwise stated. The data were also evaluated using a nonparametric test (Mann-Whitney rank-sum test). This evaluation did not change the conclusion. A result was considered significant when the *P* value was <0.05. A relative amount of fasting PIM of insulin-like immunoreactivity was calculated as PIM/(PIM + insulin) for comparison with previous studies. As meas-

Table 2—Fasting levels of β -cell peptides and ratio of intact proinsulin to PIM in diabetic patients and control subjects

	Control subjects		Type 2 diabetic patients	
	Lean	Obese	Lean	Obese
Intact proinsulin (pmol/l)	1.6 \pm 0.3	1.2 \pm 0.2	6.6 \pm 1.0*	7.7 \pm 2.0*
PIM (pmol/l)	5.4 \pm 1.0	6.1 \pm 0.9	19.9 \pm 2.5*	29.6 \pm 6.1*
Insulin (pmol/l)	30.1 \pm 3.6	42.9 \pm 4.5†	32.5 \pm 4.9	63.9 \pm 20.4
C-peptide (pmol/l)	517 \pm 38	593 \pm 27	598 \pm 32	893 \pm 112‡
PIM/(PIM + insulin) (%)	15.2 \pm 1.9	12.8 \pm 1.6	39.0 \pm 3.8*	35.1 \pm 4.7*
PIM/C-peptide (%)	1.0 \pm 0.1	1.0 \pm 0.1	3.4 \pm 0.5*	3.3 \pm 0.5*
Intact proinsulin/PIM (%)				
Fasting	33.0 \pm 3.1	21.8 \pm 2.7†	34.2 \pm 2.9	26.6 \pm 2.9
6-min after intravenous glucagon	22.5 \pm 1.5	15.8 \pm 1.5†	32.3 \pm 2.7*	27.9 \pm 3.6*

Data are means \pm SEM. * $P < 0.01$, † $P < 0.05$ for obese vs. lean control subjects, ‡ $P < 0.05$ for lean type 2 diabetic patients vs. lean control subjects, or obese type 2 diabetic patients vs. obese control subjects.

ures of β -cell function for the various peptides, acute incremental values and total increments above baseline (area under the curve [AUC]) were calculated according to the trapezoidal rule. The 6-min increment was calculated as the 6-min value minus the basal value.

RESULTS

Fasting values

Intact proinsulin was increased four- to sixfold in type 2 diabetes (Table 2). The ratio of intact proinsulin to PIM (intact proinsulin/PIM) was comparable between the groups; in comparing the two groups of normal subjects, however, we found this ratio to be significantly decreased in obese subjects.

PIM was significantly elevated in lean type 2 diabetic patients (LD) compared with lean control subjects (LC) and in obese type 2 diabetic patients (OD) compared with obese control subjects (OC). No significant differences were seen between

the diabetic groups or the control groups. For insulin, we found no significant difference between LD and LC or OD and OC. OC did have elevated levels compared with LC, an indicator of insulin resistance. C-peptide showed the same pattern as intact insulin; however, levels in OD were significantly higher than those in OC. PIM was disproportionately elevated [PIM/(PIM + insulin) and PIM/C-peptide] in diabetic patients, being raised 2.5- to 4-fold compared with BMI-matched control subjects. There was no significant difference among the control or the type 2 diabetic groups.

Glucagon-stimulated values

In contrast to the fasting state, we found the ratio of intact proinsulin to PIM to be significantly increased in samples obtained 6 min after glucagon stimulation (peak response) from the type 2 diabetic patients ($P < 0.01$) (Table 2). OC had, after stimulation, as in the fasting state, a significantly decreased ratio of intact proinsulin to PIM,

$P < 0.05$. We observed a two- and sixfold higher increase of intact proinsulin (6-min incremental response) in lean and obese diabetic patients versus control subjects ($P < 0.05$ and < 0.01), respectively (Table 3).

PIM levels during the test were significantly elevated in the diabetic subjects and most pronounced in the obese diabetic patients ($P < 0.05$) (Fig. 1). The groups of normal subjects were comparable. However, the net PIM responses during the test, measured as AUC, were not increased in either lean or obese diabetic patients compared with control subjects (Table 3). In contrast, the AUC for intact insulin and C-peptide were significantly reduced for both groups of diabetic patients; the reduction was most pronounced, however, in the lean group ($P < 0.01$). It is apparent from Figs. 1 and 2 that baseline levels are reached for both PIM and insulin (and C-peptide, although it is not shown) during the 120-min glucagon test. However, apparently basal levels were reached faster

Table 3—Levels of β -cell peptides after intravenous glucagon stimulation in diabetic patients and control subjects

	Control subjects		Type 2 diabetic patients	
	Lean	Obese	Lean	Obese
AUC (pmol \cdot 1 ⁻¹ \cdot min ⁻¹)				
PIM	392 \pm 51	461 \pm 74	425 \pm 56	480 \pm 82
Insulin	4,348 \pm 483	5,164 \pm 526	1,577 \pm 267*	3,218 \pm 1,008
C-peptide	32,840 \pm 1,870	39,140 \pm 4,070	16,850 \pm 2,180*	24,950 \pm 5,180†
PIM/insulin(%)	9.2 \pm 1.1	9.1 \pm 1.1	32.6 \pm 6.7*	22.7 \pm 5.2†
PIM/C-peptide(%)	1.2 \pm 0.1	1.2 \pm 0.1	2.7 \pm 0.3*	2.2 \pm 0.4†
6-min increment (pmol/l)				
Intact proinsulin	1.8 \pm 0.5	1.0 \pm 0.2	4.0 \pm 0.9‡	6.1 \pm 1.3§

Data are means \pm SEM. AUC data are from 0–120 min after intravenous glucagon stimulation. 6-min increment responses are from after intravenous glucagon stimulation: 6-min value minus fasting value. * $P < 0.01$, † $P < 0.05$ for obese type 2 diabetic patients vs. obese control subjects, ‡ $P < 0.05$ for lean type 2 diabetic patients vs. lean control subjects, § $P < 0.01$.

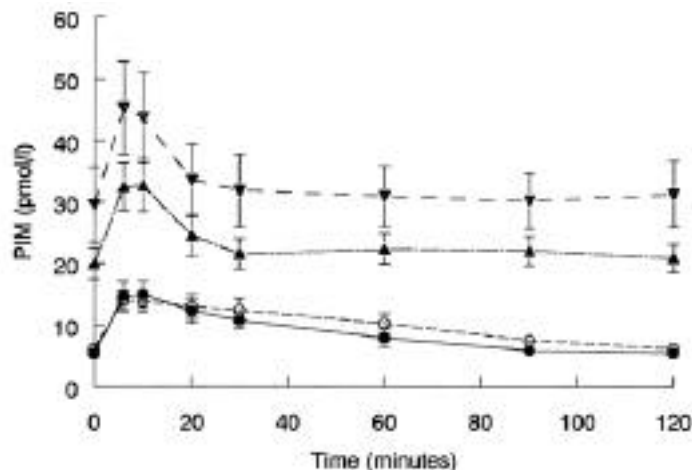


Figure 1—Time course of PIM during the 120-min glucagon test (1.0 mg i.v.). ●, LC; ○, OC; ▲, LD; ▼, OD.

in the diabetic patients; i.e., the duration of the responses seem to be three to four times shorter.

The ratio of PIM to insulin or C-peptide during the test (calculated as AUC ratios) was significantly elevated in both lean and obese diabetic patients; it was most pronounced, however, for the lean group, with ratios elevated 2.3- to 3.5-fold versus lean control subjects, whereas the OD had elevations of 1.8- to 2.5-fold versus the OC (Table 3). The difference in PIM/insulin or PIM/C-peptide between diabetic patients and control subjects during the test was within the same magnitude as that observed in the fasting state.

CONCLUSIONS — In this study, we observed by specific immunoassays that intact proinsulin levels were higher and the ratio of intact proinsulin to PIM was disproportionately elevated after glucagon stimulation in type 2 diabetic patients. These results are consistent with the elevated ratio of proinsulin-to-insulin (or C-peptide) in type 2 diabetes observed in many previous studies (8–10,14,28,29), being caused by elevated levels of intact proinsulin as well as proinsulin conversion intermediates.

This abnormality does not seem to be caused or affected by obesity. No difference was observed in the levels of intact proinsulin or PIM between lean and obese subjects with normal glucose tolerances, either under fasting (steady-state) conditions or in response to a nonglucose β -cell secretagogue. Surprisingly, we observed an apparent decrease in the ratio of intact proinsulin to PIM in obese subjects in both situations. In patients with β -cell dysfunction (type 2

diabetes) intact proinsulin levels were comparable, with and without concomitant obesity, both in absolute and in relative terms. Our two groups of type 2 diabetic patients were carefully matched for degree of hyperglycemia. We may therefore conclude that adding an additional secretory demand on the β -cells in obesity-associated (modest) insulin resistance does not cause a further increase in intact proinsulin. A disproportionate increase in intact proinsulin levels after acute stimulation with glucagon suggests that intact proinsulin may be released in increased amounts in type 2 diabetes. Responses from the prolonged glucagon test show, by use of measures for intact insulin and C-peptide, that β -cell function is clearly deficient in type 2

diabetes. However, PIM responses were not altered in the type 2 diabetic groups. The apparent reduction in duration of PIM, insulin, and C-peptide responses could be due either to a decreased stimulatory effect of glucagon in terms of duration in type 2 diabetic patients or to a maintained response in the control subjects to raise glucose levels (~1–2 mmol/l in our study) because of the glucagon effect. The glucose effect would not be expected in the diabetic patients (2,3).

To our knowledge, no previous study has demonstrated a disproportionate increase in intact proinsulin in type 2 diabetes and a decrease in obesity. There may be two reasons for this: assay methodology and match for adiposity and glycemia. Other current immunoassays for proinsulin immunoreactivity are measuring a sum of intact proinsulin and the conversion intermediates with varying efficiency (22,30–33) or are more or less specific assays for intact proinsulin (15,34–36), but all with some degree of cross-reactivity from des(64,65)- or des(31,32)-proinsulin. By using such methods, Temple et al. (28) found raised intact proinsulin in both lean and obese type 2 diabetic patients, but also increases in obese normal subjects. They found somewhat higher levels for intact proinsulin compared with those found in our study. In another study, comparable levels of intact proinsulin were found in type 2 diabetic patients with mild hyperglycemia (36), but with a significant overlap between diabetic patients and normal subjects. In our study, only a minor overlap

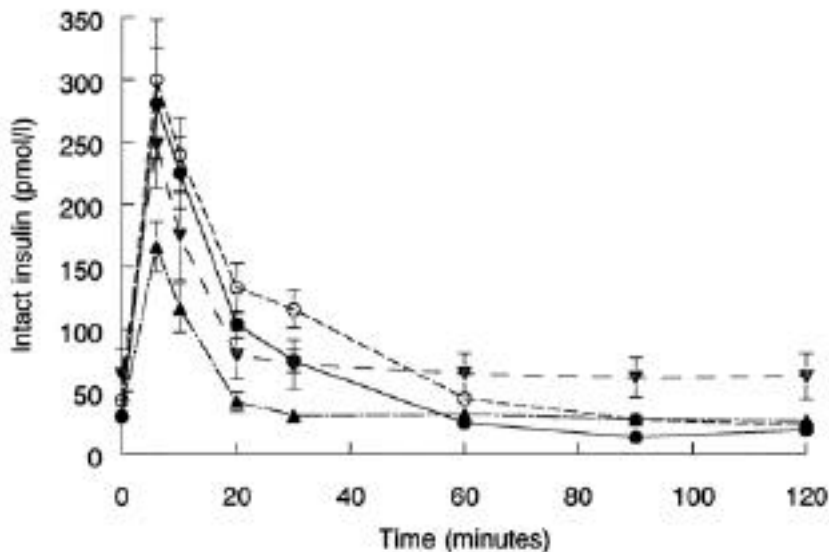


Figure 2—Time course of intact insulin levels during the 120-min glucagon test (1.0 mg i.v.). ●, LC; ○, OC; ▲, LD; ▼, OD.

was found for fasting intact proinsulin levels between control subjects and diabetic patients; thus only three values in the diabetic patients were in the normal range: 1.7, 2.0, and 3.5 pmol/l.

Recent HPLC methods have been successful in discriminating between intact proinsulin and its four conversion intermediates (16,17). We found previously by HPLC the composition of proinsulin immunoreactivity to be ~60:40% des(31,32)-proinsulin:intact proinsulin (10) for both type 2 diabetic patients and normal subjects, a somewhat higher proportion of intact proinsulin than that found in this study. This was in accordance with another HPLC study (37). Why the effect of adiposity should be to lower intact proinsulin levels is uncertain. It may be speculated that the extra demand, due to decreased insulin sensitivity, may stimulate the activity of the conversion enzymes in face of normal β -cells. An increased clearance is not likely, since there was no tendency toward increased clearance of PIM in obese subjects during glucagon testing (Fig. 1).

Our observations have some previous experimental and clinical support. Kahn et al. (11) found a modest decrease in proinsulin-to-insulin ratio when inducing insulin resistance by nicotinic acid in streptozotocin-treated baboons. Neither we (38) nor others (39) found any effect on the proinsulin-to-C-peptide ratio when acutely challenging normal subjects with hyperglycemia (8 and 3 h). Long-term hyperglycemia, causing glucose toxicity in the β -cells, may have differential effects. In type 2 diabetic patients, there seem to be an effect on the proinsulin-to-insulin ratio and on intact proinsulin when β -cell demand from hyperglycemia is relieved by introducing dietary treatment (40) and metformin treatment (41), but no effect was observed when sulphonylurea treatment (glibenclamide or gliclazide) was introduced (42,43).

Our results suggests that intact proinsulin is disproportionately elevated in type 2 diabetes. This increase and a disproportionate elevation in total proinsulin are not explained by secondary effects caused by obesity. The pancreatic β -cell response to glucagon seems to be both quantitatively and qualitatively abnormal in type 2 diabetes.

The authors would like to thank Susan Kjellberg and Quan Truong for excellent technical assistance. Levels of islet cell antibodies were kindly measured by Dr. Mikael Knip, University of Oulu, Finland.

This study has been published previously in abstract form at the European Association for the Study of Diabetes meeting in Stockholm, 1995.

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Acknowledgment— This work was supported by a grant from the Juvenile Diabetes Foundation International.

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