CONTRIBUTION OF TOTAL AND INTACT PROINSULINS TO HYPERINSULINISM IN SUBJECTS WITH OBESITY, IMPAIRED GLUCOSE TOLERANCE OR TYPE 2 DIABETES

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SUMMARY - The recent development of specific radioimmunoassays of insulin (Ins) and proinsulin (PI) led some authors to question the classical data on insulin secretion in patients with abnormal glucose tolerance. The aim of this work was to determine the participation of intact proinsulin (iPI) and its split fragments to total insulin secretion in obese subjects and in various stages of glucose intolerance determined by an oral glucose load according to the WHO recommendations. Five groups were constituted: non obese controls (C), obese subjects with normal glucose tolerance (O), non obese subjects with impaired glucose tolerance (I), obese subjects with impaired glucose tolerance (DI) and diabetic patients (D). The basal level of total Proinsulin (tPI) of D and DI was significantly higher than that of C, but the tPI/Ins ratio did not differ between the five groups. After glucose load, this ratio tended to be higher in D, but not significantly. No statistical difference between groups was observed for the iPI/Ins ratio. These results indicate that the determination of tPI is at least as informative or more than that of iPI. Furthermore, the proportionally constant participation of PI to insulin secretion observed in various stages of glucose intolerance suggests that the results obtained in the past with non specific insulin radioimmunoassays remain valid.

Key-words: specific insulin, total proinsulin, intact proinsulin, impaired glucose tolerance, obesity, diabetes.

RÉSUMÉ - Participation de la proinsuline totale et intacte à l’hyperinsulinisme de sujets obèses, intolérants au glucose et diabétiques de type 2

La mise au point récente de dosages de l’insulinémie vraie et de la proinsulénémie a conduit certains auteurs à remettre en question certaines notions classiques sur l’insulinosécrétion dans les états d’intolérance au glucose. Ce travail avait pour but de déterminer la participation de la proinsuline et de ses fragments de clivage à l’insulinosécrétion globale au cours de l’obésité et de divers stades d’intolérance au glucose, déterminés par une épreuve de charge orale en glucose réalisée selon les recommandations de l’OMS. Cinq groupes ont été constitués: témoins de poids normal (T), obèses normotolérants au glucose (O), intolérants au glucose de poids normal (I), intolérants au glucose obèses (IO) et diabétiques (D).

A l’état basal, la proinsuline totale (PI tot) des D et des IO est significativement supérieure à celle des T, mais le rapport PI tot/Ins reste identique dans les différents groupes. Après charge orale en glucose, ce rapport tend à être supérieur chez les D mais non significativement. Aucune différence statistiquement significative n’est observée pour la mesure de la PI intacte ou le rapport PI int/Ins.

Il apparaît donc que le dosage de la PI totale est au moins aussi informatif voire davantage que celui de la PI intacte. D’autre part, la participation proportionnellement constante de la PI à l’insulinosécrétion observée au cours de divers stades d’intolérance au glucose suggère que les résultats obtenus avec des techniques non spécifiques de dosage de l’insuline restent valides.

Mots-clés : insulin spécifique, proinsuline totale, proinsuline intacte, obésité, intolérance au glucose, diabète.

Most studies on the pancreatic beta-cell function in subjects with various glucose tolerance (GT) troubles were carried out with conventional insulin competitive radioimmunoassays (RIAs). RIAs cross-react with proinsulin (PI) and its intermediate metabolites: split 32,33, des 31,32 proinsulins and split 65,66, des 64,65-proinsulins. So that immunoreactive insulin (IRI) values measured with RIAs may have led to overestimate hyperinsulinism in patients with impaired glucose tolerance [1]. In fact, intact proinsulin (iPI) and des 31,32 proinsulins are the major circulating forms of PI and represent more or less total proinsulin (tPI), the concentration of des 64,65 PI being extremely low [2].

The use of monoclonal antibodies in non competitive immunometric assays has allowed to develop assays able to measure the concentrations of insulin, iPI and total tPI with a good specificity and sensitivity. These assays are no longer confined to research laboratories [3] but are now commercially available and may be used routinely.

This study was performed to determine the contribution of tPI and iPI to the insulin secretion in subjects with obesity, impaired glucose tolerance or newly diagnosed compensated type 2 diabetes.

### SUBJECTS AND METHODS

#### Patients

We analysed sera obtained from 71 subjects during a 3-h oral glucose tolerance test (OGTT) using 75g of glucose. All patients gave their informed consent. The group of patients included obese subjects, subjects with familial history of type 2 diabetes, subjects with moderate fasting hyperglycemia or with suspicion of functional hypoglycemia. Glucose, insulin, iPI and tPI levels were measured in fasting subjects and subsequently one, two and three hours post glucose load.

As a function of their body mass index (BMI), the subjects were classified as obese (BMI ≥ 28) or non-obese (BMI < 28). This cut off point was chosen because it roughly represents the mean between the upper limit of normal and 30 defining obesity. As a function of their 2-h glucose concentration [6], they were considered as having a normal glucose tolerance (NGT, glucose ≤ 140 mg/dl), an impaired glucose tolerance (IGT; 140 < glucose < 200 mg/dl) or to suffer from type 2 diabetes (glucose ≥ 200 mg/dl). 5 groups were constituted: nonobese with NGT controls (C), obese with NGT (O), nonobese with IGT (I), obese with IGT (OI) and diabetic patients (D). The characteristics of the various groups are shown in Table I.

### Assays

The insulin (ins) level was assayed with an immunoradiometric kit (Bi-Insuline from Sanofi Diagnostics Pasteur, France). The detection limit was 1.2 pmol/l and interassay CVs were below 8%. This assay has a negligible cross-reactivity with PI and split 32,33 or des 31,32 PI and 100% cross-reactivity with split 65,66 and des 64,65 PI [4].

iPI and tPI were assayed with ELISA kits (Dako, UK). The antibodies used in these kits and prototypes of the assay have been previously described [3, 5]. The detection limits are 0.07 pmol/l and 0.13 pmol/l, respectively; interassay CVs below 8 and 9%. The iPI assay has a negligible cross-reactivity with split 32,33 and des 31,32 PI and a cross-reactivity with split 65,66 and des 64,65 PI ranging between 44 and 73%.

| Table I. Basic characteristics of subjects in the 5 groups: nonobese with NGT (C), obese with NGT (O), nonobese with IGT (I), obese with IGT (OI) and type 2 diabetic patients (D). |
|---|---|---|---|---|
| C | O | I | OI |
| N | 24 | 9 | 12 | 13 | 13 |
| Sex M/F | 16/8 | 6/3 | 8/4 | 6/7 | 5/8 |
| Age (years) | 43.3 ± 3.8 | 51.1 ± 5.9 | 55.5 ± 4.1 | 59.1 ± 2.8 | 57.7 ± 4.2 |
| Range (years) | 17-79 | 28-71 | 26-72 | 45-71 | 38-76 |
| BMI (kg/m²) | 22.5 ± 0.54 | 30.7 ± 1.0 | 24.0 ± 0.85 | 34.0 ± 1.5 | 28.6 ± 1.4 |
| Range (kg/m²) | 17.8-27.1 | 28.1-36.2 | 19.0-27.4 | 28.0-44.4 | 18.4-35.2 |
| 2-h glucose (mg/dl) | 110 ± 4 | 114 ± 6 | 153 ± 5 | 159 ± 6 | 232 ± 8 |
| Range (mg/dl) | 79-140 | 77-140 | 142-176 | 148-198 | 203-272 |

Data are given as means ± SEM.
The tPI assay cross-reacted between 68 and 100% with the four intermediate PI metabolites. Neither of these two PI assays cross-reacted with insulin or C-peptide.

Data analysis
For descriptive and data analysis we used the Statistica software (StatSoft). The groups were compared using ANOVA completed by a post-hoc test of least significant difference. The statistical significance was set at 0.05 or less.

RESULTS
The results obtained for the 5 groups are shown in Table II.

Glucose
The fasting glucose level in diabetic patients (mean ± SD; 118 ± 16 mg/dl) was significantly higher than in the subjects in groups C (p<10⁻⁶), OI (p = 2.10⁻⁴), I (p<10⁻⁶) and O (p =2.10⁻³). Fasting glucose levels in groups O and I did not significantly differ, but it was higher in group OI than in groups C (p<0.002) and I (p<0.01).

Insulin
The fasting insulin level was higher in groups D and OI than in group C (p<0.02 and p<0.001, respectively) and than in group I (p<0.01 and p<0.001, respectively). Compared with group C, the fasting insulin was not different in group I. The increase observed in group O was borderline with the significance threshold (p = 0.056).

After glucose load, the insulin response was parallel in groups O and C, but higher in group O, the difference being significant in the 1-h sample only (436 ± 209 pmol/l vs 280 ± 164 pmol/l, p<0.05). The insulin response was normal in group I. In OI patients, the insulin response was increased and delayed compared with patients of groups C, O and I. That of patients in group D was still further delayed and increased compared to groups C and I at 2-h and 3-h.

Total proinsulin
The fasting tPI level was not significantly different in groups O and C. Patients in groups OI and particu-

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose mg/dl</th>
<th>Insulin pmol/l</th>
<th>Total proinsulin (tPI) pmol/l</th>
<th>Intact Proinsulin (iPI) pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-h</td>
<td>1-h</td>
<td>2-h</td>
<td>3-h</td>
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<tr>
<td>C</td>
<td>88</td>
<td>132</td>
<td>111</td>
<td>89</td>
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<td>±2</td>
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<td>±4</td>
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<td>O</td>
<td>92</td>
<td>151</td>
<td>114</td>
<td>89</td>
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<td>±4</td>
<td>±11</td>
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<tr>
<td>I</td>
<td>88</td>
<td>167</td>
<td>153</td>
<td>113</td>
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<td></td>
<td>±2</td>
<td>±8</td>
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<td>101</td>
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<td>120</td>
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<td>±4</td>
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<td>216</td>
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Data are given as means ± SEM.

larly D had greatly increased fasting tPI levels compared with those of group C (p<0.05 and p<0.005 respectively). The basal tPI/ins ratio did not significantly vary between groups (Fig. 1).

After glucose load, the 2-h and 3-h tPI responses in groups OI and D were higher than in group C (p<0.01). The tPI response in group I did not differ from that of group C. The tPI response in group O was significantly increased only at 2-h (p<0.03). Compared with the basal value, the tPI/ins ratio decreased in all groups at 1-h and increased subsequently (Fig. 1). Compared with all the other groups this ratio was increased in group D at 1-h only (p<0.05).

**Intact proinsulin**

Similar results were obtained for iPI, but the differences at 2-h or 3-h were not significant in D but only in OI. The iPI/ins ratios did not differ in any group at any time (Fig. 2). Compared with groups C and OI, the tPI/iPI ratio was increased in group D (p<0.05) 1-h, 2-h and 3-h after glucose load (Fig. 3).

**DISCUSSION**

On the basis of the results of several cross-sectional studies, mainly performed in populations with high risk of diabetes, it is generally admitted that basal insulin levels and the insulin response to an oral glucose load are related to fasting glycemia by a relationship resembling an inverted “U”, the so-called Starling’s curve of the β-cell [7]. Basal and post-stimulative hyperinsulinemia is usually observed in patients with impaired glucose tolerance, while type 2 diabetes is considered as a result of the inability of the pancreatic β-cell to compensate for insulin resistance. Elevated insulin levels, in absolute terms, can be observed at the beginning of the disease, but patients become truly insulinopenic as plasma glucose rises. However, the respective role of obesity and diabetes has been discussed, and these results, based on non specific radioimmunoasays, were questioned by the observation in type 2 diabetics of an unexpectedly high proportion of proinsulin and proinsulin conversion products [1], which normally count for only 10 to 20% of total insulin immunoreactivity. This high proportion of PI has been attributed to an abnormal PI processing and/or secretion [8], or to the increased demand on the β-cell due to insulin-resistance or hyperglycemia [9]. According to these results, Temple et al. suggested that all diabetics were in fact hypoinsulinemic. Several others reported high PI/ins ratios in type 2 diabetics [2, 10-12], and less frequently in patients with impaired glucose tolerance [13]. This PI immunoreactivity consisting mainly on intact proinsulin and des 31-32 proinsulin, has been correlated with fasting hyperglycemia [8] and the degree of β-cell secretory capacity [10]. However, these results have generally not been confirmed in glucose intolerant or mild diabetic patients [14-17].
In a previous study comparing the results obtained with a specific insulin immunoassay and a conventional non specific one, we didn’t observe any increase of the estimated proportion of proinsulin like material in the global insulin immunoreactivity of glucose intolerant patients and compensated type 2 diabetics [18].

The aim of the present study was to directly evaluate the participation of proinsulin and its conversion products to the global insulin secretion of obese, glucose intolerant and patients with newly diagnosed type 2 diabetes.

Early sampling has been omitted because the early insulin response had been studied in our previous work and does not appear relevant for the evaluation of PI secretion considering its pharmacokinetic characteristics.

Sensitive and specific insulin and proinsulin assay methods, which are now commercially available, were
used in this study. The immunoradiometric assay of insulin does not show any significant cross-reactivity with the major circulating forms of proinsulin. The 100% cross-reactivity with split 65,66 and des 64,65 proinsulin conversion fragments has only a neglectable influence on the insulin results because of the very low concentrations of these peptides in serum [2]. The iPI assay used is considered as specific to intact proinsulin and the tPI assay is regarded as measuring iPI and the significant circulating intermediate metabolites. Our iPI results in groups C and D are in close agreement with those obtained with a proinsulin assay showing no cross-reactivity with the four proinsulin conversion intermediates [19].

The results obtained with these sensitive and specific assays confirm the classical data about insulin secretion in obese subjects and in patients with impaired glucose tolerance or recent type 2 diabetes without fasting hyperglycemia. In agreement with other data [20], insulin and proinsulin increased after a glucose load in nonobese NGT subjects, but the increase in proinsulin was delayed in comparison to insulin, with a maximum at 2-h. Insulin secretion did not differ between control and nonobese IGT subjects. That of obese IGT patients was higher than that of obese NGT patients 2-h and 3-h after glucose load, but not before. In our diabetic patients, the fasting glucose level was only moderately increased and true fasting hyperinsulinaemia was found, confirming other results [8]. The insulin response was delayed and increased compared to normal-weight or obese NGT subjects 2-h and 3-h after glucose load.

As opposed to other published results, the relative contribution of proinsulin to fasting concentrations of insulin-like molecules in obese NGT, obese and nonobese IGT [13] and diabetic patients [10-12] seemed to carry moderate weight in our study. However it must be stressed that the diabetics enrolled in these studies were all frankly hyperglycemic. This finding is in agreement with our previous indirect study in NGT, IGT and diabetic patients [18]. Our results are also consistent with those of other authors [14-16] who showed that hyperinsulinaemia in obese and IGT subjects is due to an increased secretion of both insulin and proinsulin.

The evolution of the tPI/ins ratio during OGTT reflects the difference between the half-lives of insulin (5-10 min) and proinsulins (20-30 min). A simulation using a simple mass balance model of insulin and proinsulin concentrations during an OGTT predicts that this ratio should transiently decrease, pass through a minimum later and increase above the initial value [21], as it is experimentally observed. In diabetic patients, this ratio is increased before and 1-h after glucose load, the increase being significant only 1-h after the load. This increase only concerns tPI but not iPI, suggesting that the intermediate metabolites mainly contribute to this result. This moderate increase should not call into doubt previous epidemiological and physiopathological studies which used non-specific insulin measurements.

**CONCLUSION**

Compared with that observed in normal-weight NGT subjects, the contribution of proinsulin and insulin to insulinemia, as it could be measured with a non specific insulin assay, is similar in obese NGT, obese or nonobese IGT subjects. It is only increased after a glucose load in newly diagnosed diabetic patients with near normal fasting glucose levels.

This increase seems to be rather due to incompletely processed proinsulin than to intact proinsulin. According to these results, a total proinsulin assay appears at least as informative or even more than an intact proinsulin assay.

Furthermore, our results do not question the data about insulin secretion studies, obtained with non specific insulin assays cross-reacting with proinsulin and intermediate metabolites of proinsulin.

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