agnosis, especially as there were 25% reverting to normal glucose tolerance. This reversion would appear to be due to natural fluctuations in glucose concentrations, and not to be due to a change in diet or medication, as the men had no knowledge about the results of their initial examination.

Our data, from the Paris Prospective Study [3], are shown in Table 1. This study involved 44–55 year-old working men, the time between the two 75 g OGTTs was on average two and a half years (between 1.2 and 4.3 years), and the WHO criteria [2] were used to define IGT and diabetes. The second OGTT was not performed on those who had been identified as being diabetic by their general practitioner, before the second OGTT. There were 26 (0.5%) such new diabetic patients from those classed normal glucose tolerant, 12 (2%) from those classed IGT and 15 (12%) from those classed diabetic on the first OGTT. Our results are strikingly similar to those of the Swedish study, both in those diagnosed IGT and those diagnosed diabetic at the first examination, despite the different time delays between tests, and the different glucose loads used.

The results on the diagnosis of IGT are similar to those reviewed by Yudkin et al. [4]: for subjects having a repeated OGTT within one year, 21–56% remained IGT, and 3–16% developed diabetes; for an OGTT repeated within 1 to 12 years, between 20–50% remained IGT, 10–47% became diabetic. Yudkin et al. suggested that insulin should be measured in IGT subjects, to discriminate between those who deteriorate to diabetes who have a failing Beta-cell function, and those who remain IGT, with persistent hyperinsulinaemia. Two reports on the risk factors for deterioration of IGT subjects to diabetes, have shown that such IGT subjects already have a diminished 2 h insulin response to a glucose load [5, 6]. The second of these reports was on the Paris Prospective Study. If we look at this idea in a very simplistic, univariate fashion for the 100 subjects in the Paris Prospective Study who were initially diagnosed as diabetic by the OGTT, this trend was also apparent, though the difference was not always significant. For fasting insulin concentrations: 155 ± 20 pmol/l (mean ± SEM) for those reverting to IGT, 146 ± 14 pmol/l for those remaining diabetic, NS for the 2 h insulin concentrations: 609 ± 59 pmol/l for those reverting to IGT, 437 ± 41 pmol/l for those remaining diabetic, $p < 0.01$.

The measurement of blood glucose is itself subject to analytic error [7]. Depending on the laboratory method used, the coefficient of variation can be as high as 8%, thus a concentration of 8 mmol/l lies somewhere between 6.7 and 9.3 mmol/l with a probability of 95%.

The most recent 'Position Statement' on the screening for diabetes, from the American Diabetes Association [8] suggested that the risk factors of the individual should be used concurrently with their glucose tolerance. These risk factors are (1) family history of diabetes (2) obesity (3) race (4) age (5) previous diagnosis of IGT (6) hypertension or significant hyperlipidaemia. In the Paris Prospective Study, for the group initially classed IGT, none of the mean values of these factors differed significantly for subjects later classified as diabetic, (either by the second OGTT or by their general practitioner), in comparison with those who remained IGT. The same was the case for those initially classed as diabetic.

The OGTT should be used with caution when diagnosing subjects either as IGT or diabetic. It was developed partly on the basis of epidemiological arguments, and a satisfactory solution to the diagnosis of diabetes in individuals must still be sought.

Table 1. Classification of subjects as normoglycaemic, impaired glucose tolerant (IGT) and diabetic according to WHO criteria following a first and a second oral glucose tolerance test (OGTT)

1st OGTT	Normo	2nd OGTT IGT	Diabetic	Total
Eriksson and Lindgärde [1], OGTTs conducted within one month				
Normoglycaemic	426 (88%)	52 (11%)	7 (1%)	485
IGT	221 (63%)	109 (31%)	23 (7%)	353
Diabetic	13 (25%)	15 (29%)	23 (45%)	51
Total	660 (74%)	176 (20%)	53 (6%)	889
Paris Prospective Study data [2], OGTTs conducted within $2^1/_2$ years, on average				
Normoglycaemic	4645 (96%)	174 (4%)	27 (0.6%)	4846
IGT	333 (69%)	127 (26%)	26 (5%)	486
Diabetic	30 (28%)	32 (29%)	47 (43%)	109
Total	5008 (92%)	333 (6%)	100 (2%)	5441

Yours sincerely,
B. Balkau and E. Eschwège

References

1. Eriksson KF, Lindgärde F (1990) Impaired glucose tolerance in a middle-aged male urban population: a new approach for identifying high-risk cases. Diabetologia 33: 526–531
2. WHO Study Group (1985) Diabetes mellitus. WHO Tech Rep Series N° 727, WHO Geneva
3. Ducimetière P, Eschwège E, Papoz L, Richard JL, Claude JR, Rosselin GE (1980) Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia 19: 205–210
4. Yudkin JS, Alberti KGMM, McLarty DG, Swai ABM (1990) Impaired glucose tolerance. Is it a risk factor for diabetes or a diagnostic ragbag? BMJ 301: 397–402
5. Sicree RA, Zimmet PZ, King HOM, Coventry JS (1987) Plasma insulin response among Nauruans. Prediction of deterioration in glucose tolerance over 6 yr. Diabetes 36: 179–186
6. Charles MA, Fontbonne A, Eschwège E (1988) Risk factors of Type 2 (non-insulin-dependent) diabetes in a Caucasian population. Diabetologia 31: 479 (Abstract)
7. Burrin JM, Alberti KGMM (1990) What is blood glucose: can it be measured? Diab Med 7: 199–206
8. American Diabetes Association (1990) Screening for diabetes. Diabetes Care 13: 7–9

B. Balkau
INSERM U21
16 ave Paul-Vaillant-Couturier
F-94807 Villejuif Cedex
France

Proinsulin conversion intermediates: a possible source of confusion

Dear Sir,

In their recent paper [1], Yudkin et al. describe the measurement of proinsulin, insulin and a major proinsulin conversion intermediate in the circulation of Type 2 (non-insulin-dependent) diabetic subjects. May I draw the attention of your readers to a possible source of confusion with regard to the identity of the conversion intermediate, in both the title and the text of this paper. Throughout this and previous papers [2, 3], the authors refer to "32–33 split proinsulin". Such an intermediate does indeed arise during proinsulin conversion as a result of an endoproteolytic attack C-terminal to Arg 32 (i.e. at the B-chain/C-peptide junction) [4]. It is, however, generally accepted that within Beta-cell granules (where conversion arises [5]) residual C-terminal basic amino acids left from such an endoproteolytic event are rapidly removed by carboxypeptidase H [6, 7]. The major conversion intermediates found in the Beta cell, or indeed in the circulation, have thus been shown to be *des* 31,32- or *des* 64,65-split proinsulin (the latter being generated by cleavage at the C-peptide/A-chain junction followed by carboxypeptidase H trimming) [7, 8]. Of these two products, it is the *des* 31,32-form which predominates [8].

The authors have themselves stressed [2] that their analytical technique, although indeed sensitive and specific [2], cannot de-

scriminate between split proinsulins and their didesamino derivatives. As mentioned above, it seems more than likely that they are in fact measuring *des* 31,32-split proinsulin (commonly referred to as *des* 31,32 proinsulin [7, 8]) and not, as they imply, split 32–33 proinsulin from which it is derived. Analysis of circulating proinsulin forms by reversed phase HPLC would resolve the issue [8]. Even if their biological potencies have been shown to be similar [9], these two intermediates are, as stressed above, discrete chemical entities reflecting different steps in the proinsulin conversion cascade. Any confusion between the two could lead to erroneous interpretation of data and is as such not simply a question of semantics. This is particularly true at a time when attention is becoming focussed on the precise molecular events involved in proinsulin processing [7, 10, 11] and on attempting to account for the hyperproinsulinaemia frequently encountered in diabetic states [1, 3, 12–15].

Yours sincerely,
P. A. Halban

References

1. Nagi DK, Hendra TJ, Ryle AJ, Cooper TM, Temple RC, Clark PMS, Schneider AE, Hales CN, Yudkin JS (1990) The relationships of concentrations of insulin, intact proinsulin and 32–33 split proinsulin with cardiovascular risk factors in Type 2 (non-insulin-dependent) diabetic subjects. Diabetologia 33: 532–537
2. Sobey WJ, Beer SF, Carrington CA, Clark PMS, Frank BH, Gray IP, Luzio SD, Owens DR, Schneider AE, Siddle K, Temple RC, Hales CN (1989) Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65–66 split and 32–33 split proinsulins. Biochem J 260: 535–541
3. Temple RC, Clark PMS, Nagi DK, Schneider AE, Yudkin JS, Hales CN (1990) Radioimmunoassay may overestimate insulin in non-insulin-dependent diabetics. Clin Endocrinol 32: 689–693
4. Steiner DF, Kemmler W, Tager HS, Peterson JD (1974) Proteolytic processing in the biosynthesis of insulin and other proteins. Fed Proc 33: 2105–2115
5. Orci L, Ravazzola M, Storch M-J, Anderson RGW, Vassalli J-D, Perrelet A (1987) Proteolytic maturation of insulin is a post-Golgi event which occurs in acidifying clathrin-coated secretory vesicles. Cell 49: 865–868
6. Davidson HW, Hutton JC (1987) The insulin-secretory-granule carboxypeptidase H. Purification and demonstration of involvement in proinsulin processing. Biochem J 245: 575–582
7. Davidson HW, Rhodes CJ, Hutton JC (1988) Intraorganellar calcium and pH control proinsulin cleavage in the pancreatic β cell via two distinct site-specific endopeptidases. Nature 333: 93–96
8. Given BD, Cohen RM, Schoelson SE, Frank BH, Rubenstein AH, Tager HS (1985) Biochemical and clinical implications of proinsulin conversion intermediates. J Clin Invest 76: 1398–1405
9. Peavy DE, Brunner MR, Duckworth WC, Hooker CS, Frank BH (1985) Receptor binding and biological potency of several split forms (conversion intermediates) of human proinsulin. J Biol Chem 260: 13989–13994
10. Gross DJ, Villa-Komaroff L, Kahn CR, Weir GC, Halban PA (1989) Deletion of a highly conserved tetrapeptide sequence of the proinsulin connecting peptide (C-peptide) inhibits proinsulin to insulin conversion by transfected pituitary corticotroph (AtT20) cells. J Biol Chem 264: 21486–21490
11. Docherty K, Rhodes CJ, Taylor NA, Shennan KIJ, Hutton JC (1989) Proinsulin endopeptidase substrate specificities defined by site-directed mutagenesis of proinsulin. J Biol Chem 264: 18335–18339
12. Ward WK, LaCava EC, Paquette TL, Beard JC, Wallum BJ, Porte Jr D (1987) Disproportionate elevation of immunoreactive proinsulin in Type 2 (non-insulin-dependent) diabetes mellitus and in experimental insulin resistance. Diabetologia 30: 698–702
13. Yoshioka N, Kuzuya T, Matsuda A, Taniguchi M, Iwamoto Y (1988) Serum proinsulin levels at fasting and after oral glucose load in patients with Type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 31: 355–360
14. Heaton DA, Millward BA, Gray IP, Tun Y, Hales CN, Pyke DA, Leslie RDG (1988) Increased proinsulin levels as an early indicator of B-cell dysfunction in non-diabetic twins of Type 1 (insulin-dependent) diabetic patients. Diabetologia 31: 182–184
15. Porte Jr D, Kahn SE (1989) Hyperproinsulinemia and amyloid in NIDDM. Clues to etiology of islet β-cell dysfunction? Diabetes 38: 1333–1336

Dr. P. A. Halban
Laboratoires de Recherche Louis Jeantet
Centre Médical Universitaire
1 rue Michel Servet
CH-1211 Geneva 4
Switzerland

Announcements

23rd Journees de Diabetologie de L'Hotel-Dieu

This congress will be held on May 27–29, 1991, in Paris, France. *For more information please contact:* Ms. A. Forge, Secretariat, Hôtel-Dieu, 1, place du Parvis Notre-Dame, F-751 81 Paris Cedex 04, France. Tel: (1) 42 34 83 88, Telecopie (1) 43 54 15 64.

3rd International Symposium on Diabetic Angiopathy in Childhood

This symposium will be held on September 2–4, 1991, in Berlin, FRG. Sponsored by the International Study Group on Diabetes in Children and Adolescents (ISGD). The meeting is designed as a workshop for clinicians and scientists. *For further information and abstract forms please contact:* Dr. Bruno Weber, Universitäts-Kinder-Klinik, Heubnerweg 6, 1000 Berlin 19, FRG. Tel: 30-3035-1, Fax: 30-3035-4638.

4th International Symposium on Hypoglycemia

This symposium will be held on March 22–24, 1992, at the Istituto Superiore di Sanità, Rome, Italy. *For further information please contact:* G. Tamburrano, M. D., C.I.S.D, Via Baglivi, 12, I-00161 Rome, Italy. Tel: 0039 6 44 53 624, Fax: 0039 6 88 31 141