Fasting Proinsulin Concentrations Predict the Development of Type 2 Diabetes

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OBJECTIVE — The development of specific assays allows the different molecules in the proinsulin processing pathway to be measured separately. 32,33 Split proinsulin is the predominant form of proinsulin and accounts for the disproportionate hyperproinsulinemia seen in individuals with prevalent type 2 diabetes. This study was established to examine whether the concentration of this molecule predicts diabetes.

RESEARCH DESIGN AND METHODS — A population-based longitudinal cohort study was conducted in Ely, Cambridgeshire. At baseline, 1,122 individuals completed a 75-g oral glucose tolerance test (OGTT). At the 4.5-year follow-up study, repeat OGTTs were performed on 937 of the cohort of 1,071 individuals who had been nondiabetic at baseline.

RESULTS — A total of 26 people progressed to diabetes as determined by the OGTTs. The risk of progression was strongly related to the fasting glucose concentration (relative risk [RR] comparing top with bottom quartile 17.6 [95% CI 2.4–130.4]) and fasting 32,33 split proinsulin (RR 16.4 [2.2–121.9]), but less strongly to the fasting insulin (RR 4.41 [1.5–12.9]) or intact proinsulin (RR 5.2 [1.5–17.3]). In multivariate analyses, these associations were independent of age, sex, BMI, and baseline glucose tolerance category. Subjects in the top quartile for fasting glucose and total proinsulin with a family history of diabetes were a high-risk subgroup (incidence 65.8 per 1,000 person-years of follow-up [pyfu]); 30% of them progressed to diabetes at follow-up.

CONCLUSIONS — Fasting 32,33 split proinsulin independently predicts the development of diabetes. This prediction was better than that observed for either the insulin or intact proinsulin concentrations. The combination of family history, fasting glucose, and total proinsulin identified a subgroup of individuals at high risk of progression who might benefit from targeted interventions.

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Previous cross-sectional studies have shown that the concentration of proinsulin-like molecules (PLMs) is disproportionately increased in people with type 2 diabetes (1–3), impaired glucose tolerance (IGT) (4), and gestational diabetes (5). In prospective studies, the concentration of all PLMs and the ratio of PLMs to total immunoreactive insulin have been shown to predict deterioration in glucose tolerance (6–9). However, most assays have been unable to separate the various forms of partially processed forms of proinsulin from intact proinsulin. Because the conversion from 32,33 split proinsulin to insulin is a rate-limiting step in the proinsulin cleavage pathway (10) and 32,33 split proinsulin is the predominant form of proinsulin accounting for the disproportionate hyperproinsulinemia of diabetes (11), we hypothesized that the concentration of 32,33 split proinsulin would predict the development of type 2 diabetes.

In a cross-sectional population-based study using specific assays for intact and 32,33 split proinsulin (12), we demonstrated an increased concentration of both these molecules in individuals with previously undiagnosed diabetes and IGT (13, 14). The present study was conducted to determine whether the fasting concentration of these molecules predicted the development of diabetes in a 4.5-year follow-up of this cohort, whether they were better predictors than other insulin measures, and whether they could contribute to the identification of a high-risk subgroup.

RESEARCH DESIGN AND METHODS

The Isle of Ely Study was established in 1990 as a longitudinal cohort study of the etiology and pathogenesis of type 2 diabetes and related metabolic disorders. The study design and methods have been described in detail elsewhere (12); but in brief, a sample of 1,122 Caucasian subjects were selected at random from a sampling frame consisting of all adults free of known diabetes who were registered with a single general practice in the City of Ely (response rate 74%). Comparison of the study cohort with local population level socioeconomic and anthropometric data does not suggest any systematic differences between the subjects who agreed to participate and the population from which they were sampled. Volunteers attended for a standard 75-g oral glucose tolerance test (OGTT) and a clinical examination that included a dietary and medical questionnaire and standard anthropometric measurements. Volunteers were asked to fast from 10:00 P.M. the previous evening. Smoking status and alcohol intake were assessed using the Health and Lifestyle Survey questionnaire (15), and a detailed family history was taken. Where a parent or a sibling was reported to have diabetes, the age at onset and initial mode of treatment were ascertained. For the purposes of these analyses, a person was classified as having a positive family history if either a parent or

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Abbreviations: CV, coefficient of variation; IGT, impaired glucose tolerance; MET, metabolic equivalent; OGTT, oral glucose tolerance test; OR, odds ratio; PLM, proinsulin-like molecule; pyfu, person-years of follow-up; RR, relative risk.

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

Table 1—*Comparison of baseline characteristics by phase II follow-up status*

Phase II follow-up status	*n*	Phase I characteristics: Age (years)	BMI (kg/m²)	120-min glucose (mmol/l)
Men				
Volunteer	394	54.3 ± 7.9	26.0 ± 3.0	6.29 ± 1.6
Refusal	51	52.4 ± 7.3*	25.9 ± 3.6	5.83 ± 1.4
Exclusion	9	51.7 ± 8.9	26.2 ± 3.4	6.53 ± 1.9
Deceased	10	58.4 ± 5.8	24.8 ± 4.3	5.56 ± 1.6
Women				
Volunteer	543	53.4 ± 7.6	25.5 ± 4.7	6.31 ± 1.6
Refusal	41	52.6 ± 7.6	28.0 ± 6.9†	6.51 ± 1.5
Exclusion	13	55.0 ± 9.0	28.4 ± 5.6	7.05 ± 1.8
Deceased	10	58.4 ± 7.6	25.6 ± 5.0	6.17 ± 2.0

Data are means ± SD. *n* = 1,071. Significance levels for *t* test comparison of means with volunteers within sex, **P* = 0.07, †*P* = 0.06.

sibling was reported to have had diabetes beginning over the age of 30 and not treated by insulin in the first year after diagnosis. Leisure time physical activity was assessed using the questionnaire from Paffenbarger et al. (16) with standard coding of specific activities using published MET scores (or metabolic equivalents) for recreations and sports (17). Serum and plasma samples were obtained at fasting and 30 and 120 min postglucose load. The plasma samples were immediately separated in a cooled centrifuge at 4°C. Serum was removed from the clotted samples after 1 h. All the fasting samples were taken between 8:30 and 9:30 A.M. They were immediately placed on ice and permanently stored at −70°C within 4 h. Glucose concentrations were measured immediately in the routine laboratory using a hexokinase assay (18). Plasma insulin was measured by two-site immunometric assays with either ^{125}I or alkaline phosphatase labels (19,20). Cross-reactivity was <0.2% with intact proinsulin at 400 pmol/l and <1% with 32,33 split proinsulin at 400 pmol/l. Interassay coefficients of variation (CVs) were 6.6% at 28.6 pmol/l (*n* = 99), 4.8% at 153.1 pmol/l (*n* = 102), and 6.0% at 436.7 pmol/l (*n* = 99). Insulin concentrations were measured in fasting and 30- and 120-min samples. Plasma intact proinsulin and 32,33 split proinsulin concentrations were measured using immunoradiometric assays (19). The intact proinsulin assay shows <1% cross-reactivity with insulin and 32,33 split proinsulin at 2,500 pmol/l and 400 pmol/l, respectively. Between-batch CVs are 10.5% at 4.5 pmol/l, 8.5% at 20 pmol/l, and 8.1% at 92.9 pmol/l (*n* = 50). The 32,33 split proinsulin assay shows 87% cross-reactivity with intact proinsulin. To obtain the specific measure of 32,33 split proinsulin, it is necessary to take account of the intact proinsulin concentration. Cross-reactivity with insulin is <1% at 2,500 pmol/l. Between-batch CVs are 8.6% at 6.6 pmol/l, 6.1% at 41 pmol/l, and 5.3% at 101.2 pmol/l (*n* = 50).

After a local media campaign in 1994, the second phase of the study was begun, and all 1,071 subjects who were nondiabetic at baseline were invited to attend for repeat testing. Individuals who had moved from the Ely area were traced via their new Family Health Services Authority and general practitioner. When volunteers were found to have moved away from the Ely area, they were invited to return to Ely for testing, but when this was not possible, a time was arranged to undertake the testing either at the volunteer's general practitioner's office or in their own home. All volunteers underwent a repeat OGTT, which was conducted following the same protocol as for the phase I study. The blood samples were handled in the same manner and analyzed in the same laboratory. When subjects were studied in their own home or at a general practitioner's office outside the Ely area, blood samples were spun onsite using a portable centrifuge and were transferred to the −70°C freezer at Addenbrooke's Hospital on dry ice.

The entire cohort was flagged with the Office for National Statistics, and death certificates were obtained on all subjects within the cohort who died. When a member of the original cohort either declined to attend for repeat testing or was reported to be too sick to attend, the general practitioner's case notes were reviewed to check for an incidence of diagnosed diabetes. Both phases of the study were approved by the Cambridge Local Research Ethics Committee.

RESULTS

In phase I of the Isle of Ely Study, 1,071 subjects were found to be nondiabetic. A total of 937 of these volunteers attended for rescreening in the phase II of the study or were visited at their home or at their general practitioners' office with a mean follow-up time of 4.44 years. Of the remainder, only 92 subjects (8.6%) refused to participate in phase II of the study, 9 had moved abroad, and 13 were reported to be too ill to participate in the follow-up. The illnesses included Huntington's chorea (*n* = 1), ulcerative colitis (*n* = 1), cancer (*n* = 3), recent thrombosis (*n* = 1), severe coronary artery disease (*n* = 3), recent surgery (*n* = 2), psychiatric illness (*n* = 1), and renal failure (*n* = 1). A total of 34 of these 937 volunteers were studied outside Cambridgeshire,

Table 2—*Baseline characteristics of volunteers by baseline and follow-up World Health Organization glucose tolerance category*

Phase I glucose tolerance category	Phase II glucose tolerance category: Normal	IGT	Diabetes
Normal (*n* = 767)			
n	702	56	9
Age	52.9 ± 7.7	56.3 ± 7.4	55.2 ± 6.5
BMI	25.1 ± 3.6	27.0 ± 4.5	26.1 ± 3.5
WHR	0.81 ± 0.09	0.85 ± 0.08	0.86 ± 0.06
IGT (*n* = 170)			
n	96	57	17
Age	55.8 ± 7.7	58.6 ± 6.4	57.1 ± 6.9
BMI	27.2 ± 5.2	28.2 ± 4.4	28.4 ± 5.0
WHR	0.82 ± 0.09	0.85 ± 0.09	0.87 ± 0.12

Data are means ± SD. *n* = 937.

either in their own home or at their general practitioner's office, and 20 of the subjects who were nondiabetic at baseline died between the two phases of the study.

Although the reexamination rate for this study was high, it remained possible that those subjects who refused to attend were systematically different from those who were restudied. The baseline characteristics of subjects who were restudied were compared with those who refused to attend (Table 1). Men who refused to attend were younger than those who were restudied, and the women who did not volunteer were more overweight at baseline than those who were reexamined. However, neither of these differences reached conventional statistical significance. In men, there was a significant association between baseline smoking status and the probability of volunteering for follow-up (χ^2 = 9.9, P = 0.007). Although the same tendency for smokers to be under-represented in the follow-up was seen in women, the association was not significant.

None of the volunteers reported being diagnosed with diabetes in the 4.5 years between the two phases of the study. To check for incident disease among those that refused or were too ill to attend, we examined the general practitioner's case records of all these subjects. No cases of diagnosed type 2 diabetes were detected in either the subjects who refused to attend again or in those who were too ill to be restudied.

Table 2 shows the World Health Organization's (WHO) glucose tolerance category of the 937 subjects who were restudied by their category at the baseline study. A total of 26 new cases of type 2 diabetes were detected among the 937 subjects who were nondiabetic at the first OGTT in 1990–1992. None of these cases had been clinically diagnosed and none of the subjects had developed symptoms. A total of 17 (65%) of the incident cases of type 2 diabetes were subjects who were diagnosed as having IGT at baseline. Among those with IGT, 10% had progressed to type 2 diabetes and 33% remained IGT, but the majority (56.5%) had reverted to normal glucose tolerance. Among those with normal glucose tolerance, only 1.2% had progressed to type 2 diabetes at 4.5 years and 7.3% had developed IGT. Thus, overall in this population, the crude cumulative incidence of type 2 diabetes was 6.2 per 1,000 person-years of follow-up (pyfu) (95% CI 3.85–8.65 per

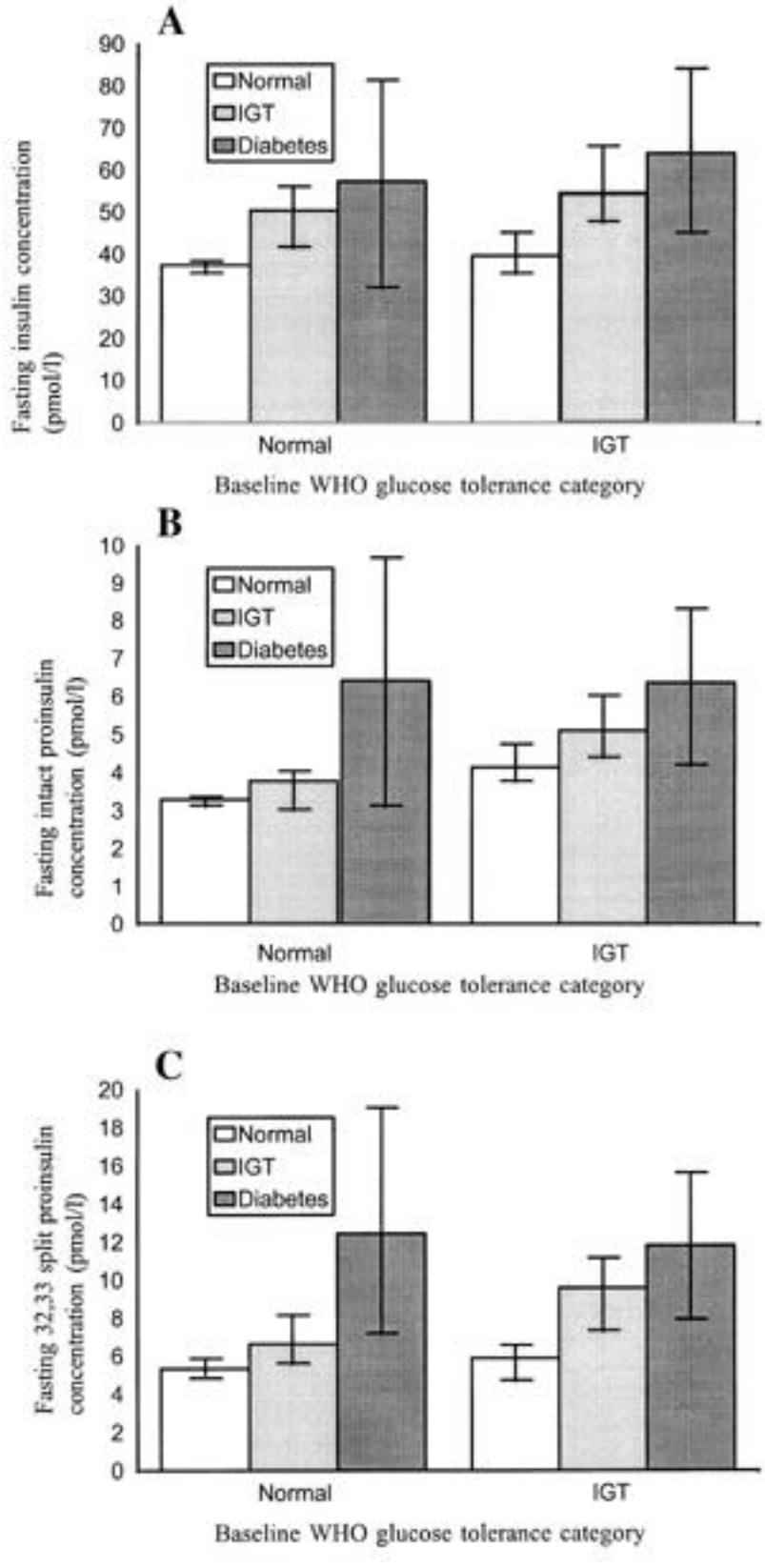

Figure 1—*Baseline geometric mean fasting insulin (A), intact proinsulin (B), and 32,33 split proinsulin (C) concentration by glucose tolerance category at 4.5-year follow-up, stratified by baseline glucose tolerance status: the Isle of Ely Diabetes Study, Phases I and II 1990–1996 (37).*

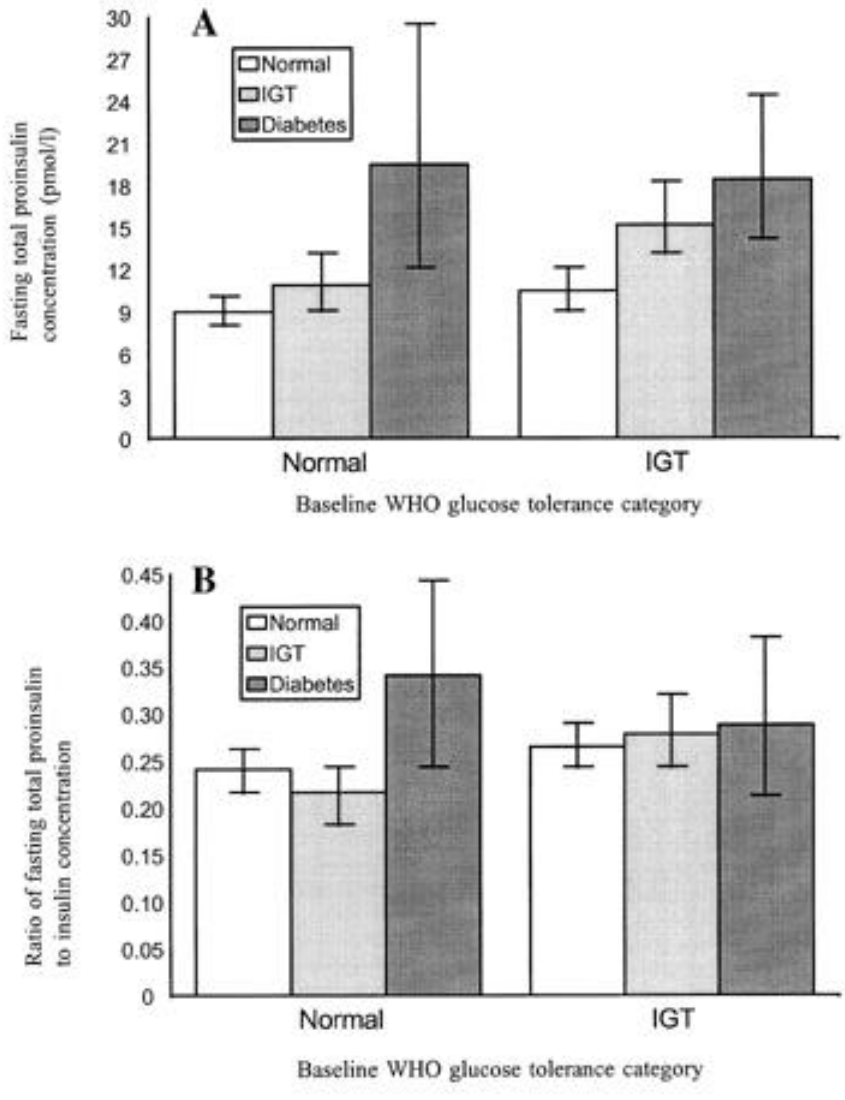

Figure 2—*Baseline geometric mean fasting total proinsulin (A) concentration and ratio of total proinsulin to insulin (B) by glucose tolerance category at 4.5-year follow-up, stratified by baseline glucose tolerance status: the Isle of Ely Diabetes Study, Phases I and II 1990–1996 (37).*

1,000 pyfu). Among subjects with IGT at baseline, the cumulative incidence of type 2 diabetes was 22.5 per 1,000 pyfu (95% CI 20.4–24.6 per 1,000 pyfu), whereas in those with normal glucose tolerance at baseline, the incidence of type 2 diabetes was 2.64 per 1,000 pyfu (95% CI 1.23–4.05 per 1,000 pyfu). Therefore, the relative risk of type 2 diabetes for subjects with IGT at baseline was 8.5 (3.9–18.8). Table 2 also shows that 56 people who originally had normal glucose tolerance progressed to IGT, an incidence rate of 16.44 per 1,000 pyfu (12.1–20.8). Because of the possibility that systematic differences existed between subjects who attended for rescreening and those who did not, we reanalyzed these incidence rates, assuming that the risk experience in those who were not rescreened could be predicted from their baseline characteristics. The greatest change in the crude incidence rate would occur for a systematic loss to follow-up, which was related to an exposure of large effect. In the case of type 2 diabetes, this situation would be most likely to occur if there was a systematic difference in baseline glucose tolerance category between volunteers and nonresponders. However, the effect of adjusting for this differential loss to follow-up was small, and the correction only reduced the incidence rate from 6.25 to 6.13 per 1,000 pyfu.

Figure 1 shows the baseline geometric mean insulin, intact proinsulin, and 32,33 split proinsulin concentrations in subjects by follow-up glucose tolerance category stratified by baseline glucose tolerance status. Subjects who progressed from normal glucose tolerance to diabetes were characterized by high fasting insulin, intact proinsulin, and 32,33 split proinsulin concentrations. However, although the CIs are wide, the relative concentration difference between the people who developed diabetes and those who remained normal or progressed to IGT was small for insulin. Whereas for both intact and 32,33 split proinsulin, the differences were much greater. In terms of the use of any biological parameter as a predictor of change, it is not only the statistical significance that is of importance, but also the magnitude of the differences between groups. Thus, the data among subjects who had normal glucose tolerance at baseline suggest that either intact or 32,33 split proinsulin would be more predictive of change than insulin. This apparent stepped-difference was not apparent in the group of subjects who originally had IGT, in whom there was a more linear increase across the follow-up categories.

Previous studies have suggested that the ratio of proinsulin to insulin may predict the development of diabetes. Therefore, Fig. 2 shows the geometric mean total proinsulin concentration and the ratio of total proinsulin to insulin by follow-up glucose tolerance category. Total proinsulin was calculated as the sum of 32,33 split proinsulin and intact proinsulin. The absolute concentration was raised in subjects who developed diabetes, irrespective of baseline glucose tolerance category. However, the pattern of the relationship with glucose tolerance category was less clear for the ratio of total proinsulin to insulin and the differences between the subjects who progressed to diabetes and those who did not were small, particularly among the subjects who had IGT at baseline.

Figure 3 shows the incidence of diabetes, expressed per 1,000 pyfu, by baseline exposure category. The risk of progressing to diabetes increased with age, such that in the group aged 55–59 years at baseline, the relative risk compared with those aged 40–44 years was 4.36 (0.94–20.18). However, the increase in incidence with age did not continue into the oldest stratum (60–65 years at baseline) in whom the relative risk compared with those aged 40–44 years was 2.61 (0.57–11.94). The incidence of diabetes also increased with BMI, but the nadir for risk was in those with a BMI between

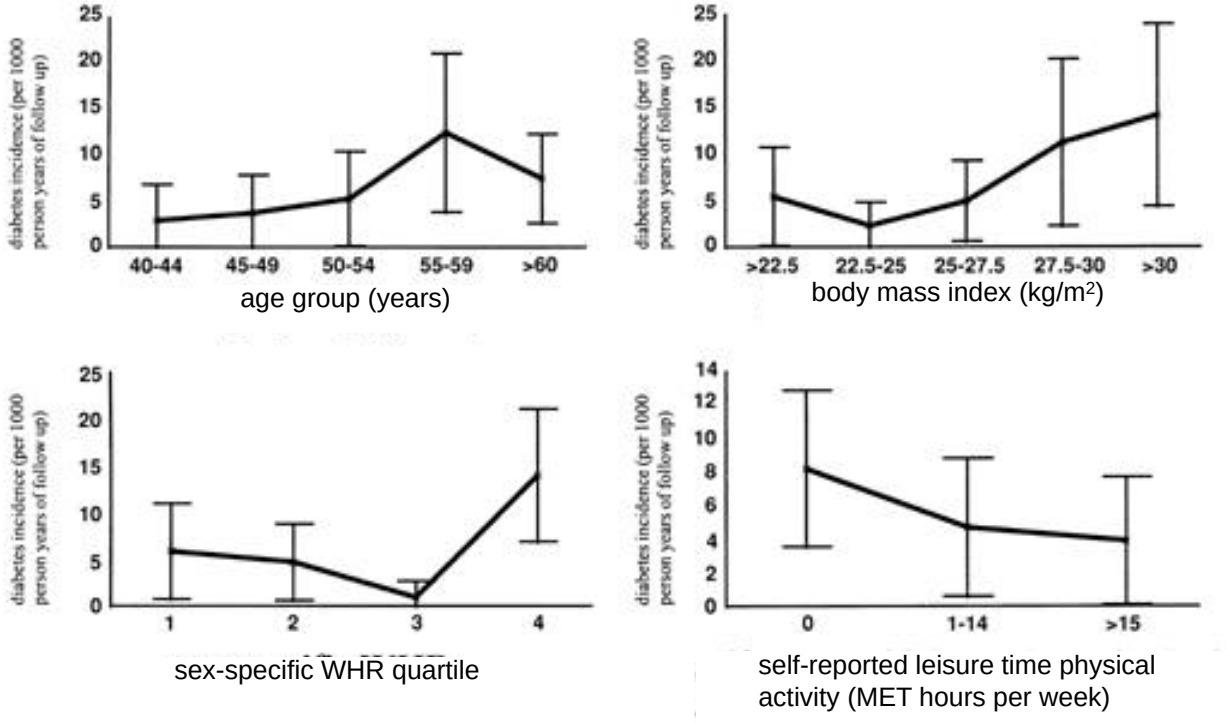

Figure 3—*The 4.5-year incidence of diabetes (per 1,000 pyfu) by baseline age-group, BMI, sex-specific waist-to-hip ratio, and category of self-reported leisure time physical activity: the Isle of Ely Diabetes Study, Phases I and II 1990–1997.*

22.5–25.0 rather than in the thinnest group (BMI <22.5). In the subjects with a BMI >30 at baseline, the relative risk compared with those with a BMI between 22.5 and 25 was 6.24 (1.68–23.13). In addition to the factors shown in Fig. 2, we examined the effect on incidence of sex and the presence of a self-reported family history of diabetes. The relative risk in men compared with women was 1.88 (0.87–4.05). A total of 172 of the 937 subjects reported having a first-degree relative with diabetes. The incidence in those with a family history was 14.21 per 1,000 pyfu (5.81–22.61), compared with 4.36 (2.15–6.56) in those with no reported family history (RR 3.26 [1.52–6.98]).

Figure 4 shows the incidence rates by quartiles for plasma glucose and the various insulin concentrations. The risk increased sharply in those with a baseline fasting glucose >6.1 mmol/l. As Table 3 shows, the relative risk comparing this group with those with baseline glucose level <5.4 was very high at 17.6. Although the risk is increased in the top quartile for both fasting insulin and intact proinsulin, the relative risk is not so large, mainly because of the nonlinearity in the lowest three quartiles (Fig. 4). However, the risk in the highest baseline fasting split proinsulin concentration was similar to that seen for the fasting glucose level at 16.4. The risk curve for the ratio of the total proinsulin to insulin shows that there is a gradually increasing risk as this ratio rises. Although this result may be of pathophysiological significance, the shape of the curve makes it unlikely that this ratio would have any use in predicting individuals at high risk of disease progression. By contrast, the very steep increase in risk that is seen for subjects in the top quartile for total proinsulin makes it possible that this measure could contribute to the prediction of individuals at high risk.

Table 4 shows the effect size per quartile for each insulin variable adjusted for age, sex, and BMI and these covariates plus baseline glucose tolerance category. In each case, there is attenuation of the effect size when baseline glucose tolerance category is considered, although all the variables remained significantly associated with the exception of the ratio of total proinsulin to insulin.

To determine the optimal model for predicting new cases of diabetes, we used backward elimination logistic regression modeling with the development of diabetes as the binary outcome variable. The variables considered by the model were age, BMI, WHR, sex, family history, physical activity category, fasting glucose, the quartiles of insulin, total proinsulin, intact proinsulin, and 32,33 split proinsulin. We did not include the ratio of total proinsulin to insulin, since this had not been an independent predictor in the multivariate analyses. As Table 5 shows, the optimal model only included three variables: the fasting glucose quartile, family history, and the total proinsulin concentration. Combining these can allow the identification of a high-risk subgroup. In subjects who have a family history of diabetes, who are in the top quartile for both fasting glucose and total proinsulin, the risk of progression to diabetes was 65.8 per 1,000 pyfu and 30% of them progressed to diabetes at the 4.5-year follow-up. The relative risk in these subjects compared with all others was 15.0 (7.2–31.4). If the top quartile of fasting glucose were used alone with family history without the proinsulin concentration, a high-risk group with an incidence of 38.5 per 1,000 pyfu would be identified, and 17% of them would progress to diabetes at 4.5 years (RR compared with all other subjects 9.0 [4.2–19.2]).

CONCLUSIONS— This study was established to examine the hypothesis that the concentration of 32,33 split proinsulin

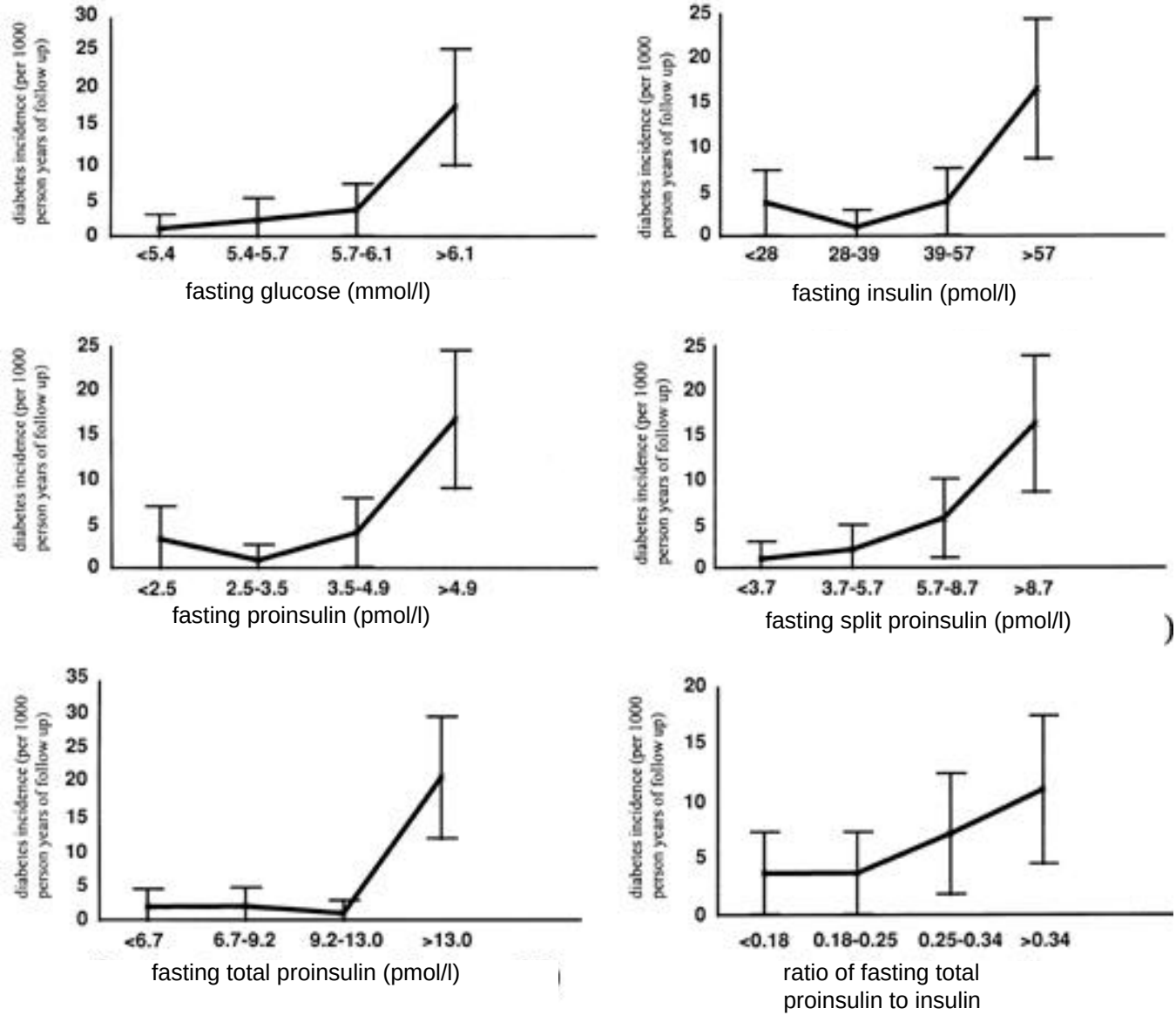

Figure 4—*The 4.5-year incidence of diabetes (per 1,000 pyfu) by quartile of baseline fasting plasma glucose, insulin, intact proinsulin, 32,33 split proinsulin, total proinsulin, and ratio of total proinsulin to insulin: the Isle of Ely Diabetes Study, Phases I and II 1990–1997 (n = 937).*

would predict the development of diabetes. The results show that subjects who progress to diabetes, irrespective of baseline glucose tolerance category, had higher fasting concentrations of insulin, intact proinsulin, and 32,33 split proinsulin. In both univariate and multivariate analyses, adjusting for age, sex, BMI, and baseline glucose tolerance category, the association with the risk of progression to diabetes was strongest for the 32,33 split proinsulin. This observation provides an opportunity not only to study the role of split proinsulin in the pathogenesis of diabetes, but also to investigate

Table 3—*Unadjusted relative risk for progression to diabetes between top and bottom quartile for baseline biochemical parameters*

	Top quartile	Bottom quartile	RR (95% CI)
Fasting glucose (mmol/l)	>6.1	<5.4	17.6 (2.4–130.4)
Fasting insulin (pmol/l)	>57	<28	4.4 (1.5–12.9)
Fasting intact proinsulin (pmol/l)	>4.9	<2.5	5.2 (1.5–17.3)
Fasting 32,33 split proinsulin (pmol/l)	>8.7	<3.7	16.5 (2.2–121.9)
Fasting total proinsulin (pmol/l)	>13	<6.7	11.1 (2.6–46.4)
Ratio of total proinsulin to insulin	>0.34	<0.18	3.0 (0.97–9.3)

$n = 937$.

Table 4—*Effect size per quartile for glucose, insulin, and proinsulin as explanatory variables in adjusted logistic regression models with incident diabetes as the outcome variable*

	Adjusted for age, sex, and BMI	95% CI	Adjusted for age, sex, BMI, and baseline glucose tolerance category	95% CI
Fasting glucose	2.83	1.60–5.01	2.34	1.29–4.23
Fasting insulin	2.09	1.28–3.18	1.92	1.21–3.03
Fasting intact proinsulin	2.18	1.34–3.53	1.95	1.20–3.18
Fasting 32,33 split proinsulin	2.44	1.47–4.06	2.27	1.35–3.81
Fasting total proinsulin	3.05	1.72–5.41	2.68	1.51–4.77
Ratio of total proinsulin to insulin	1.44	0.99–2.10	1.33	0.90–1.97

n = 937.

whether the measurement of this molecule, alone or in combination with others, could be of clinical use in identifying individuals at high risk of progressing to diabetes.

Using nonspecific assays, it has previously been shown that the fasting concentration of PLMs predicts the development of diabetes. In a nested case-control study, Haffner et al. (6) compared baseline proinsulin concentrations in 85 subjects who converted to diabetes in a 3.25-year follow-up with measurements taken in age- and sex-matched control subjects. However, the assay used to measure proinsulin in this study was relatively nonspecific and was approximate to the sum of all immunoreactive proinsulins. Similar results have been found in other populations (7–9). In the only study to have used a specific assay for 32,33 split proinsulin, Mykkänen et al. (21) using a nested case-control design showed that the fasting 32,33 split proinsulin was associated with a risk of progression when cases were compared with random control subjects (odds ratio [OR] 1.51 [95% CI 1.11–2.06]). However, when control subjects matched for sex, glucose tolerance, and BMI were used, the association was no longer significant (OR = 1.32 [0.90–1.93]). In this study, the total proinsulin to specific insulin ratio predicted deterioration, but the authors did not examine whether total proinsulin itself was predictive (21). Our study is, therefore, the first to have shown that the fasting concentration of 32,33 split proinsulin predicts progression to diabetes, although preliminary results from a study in Sweden are similar (22). The ratio of total proinsulin to insulin was a less strong predictor in our study than in that by Mykkänen et al. (21), and in multivariate analyses adjusting for age, BMI, and sex, this was not significant. The association in this study between high fasting 32,33 split proinsulin concentrations and the development of diabetes suggests that subjects with β-cell dysfunction are at high risk of disease progression.

An alternative reason for searching for predictive factors is to specify high-risk subgroups. This has a particular use in diseases such as type 2 diabetes, in which interventions may be targeted at individuals at increased risk. If the goal is to predict and if the intention is for this to be undertaken at a population level, then the main criteria for selecting factors for consideration is that they should be relatively easily measured and applicable at a population level. Therefore in establishing our prediction model, we only included simple anthropometric variables or biochemical parameters that were measurable in fasting samples. By contrast to etiological models, prediction models need only include variables that explain the majority of the variance in an outcome. Several factors, including age, sex, BMI, and physical activity, were excluded from the final model, and this may at first seem paradoxical, given what has been observed in other studies. However, these historical risk factors are all cross-sectionally linked with the fasting glucose, and their absence from a predictive model is a reflection of this shared variance and does not imply any etiological relevance. The optimal model for predicting diabetes only included three variables; a family history of diabetes, the fasting glucose, and the concentration of fasting total proinsulin defined as the sum of 32,33 split proinsulin and intact proinsulin. These factors can be combined to identify a subgroup of subjects who are at high risk of progressing to diabetes. In people who have a family history whose fasting glucose is >6.1 mmol/l, the risk of progressing to diabetes at 4.5 years was 17%. By measuring the concentration of fasting proinsulin, the risk in this subgroup can be refined, such that in the group of subjects with these two factors and a fasting total proinsulin >13 pmol/l, the relative risk compared with all other subjects rises from 9.0 to 15.0 and the absolute risk rises to 65.8 per 1,000 pyfu or 30% at 4.5 years. This is a much higher risk subgroup even than IGT, in which the absolute risk is 22.5 pyfu, and is practically easier to identify as it only uses fasting values.

The overall cumulative incidence of type 2 diabetes in this study was similar to other studies in Caucasian populations (23). Although there are an increasing number of population-based cross-sectional studies using standardized methods in which the point prevalence of diabetes and IGT has been measured (24), there are relatively few studies using repeat OGTT to produce estimates of the true incidence (25), and none of these have been conducted in Britain (26). Those studies that have measured the incidence of diabetes in

Table 5—*Optimal logistic regression model with diabetes as the outcome variable*

Independent variable	β coefficient	SEM	Significance	OR
Fasting glucose quartile	0.772	0.289	0.008	2.16
Family history	1.09	0.421	0.01	2.97
Total proinsulin quartile	0.876	0.290	0.003	2.40

n = 937. Variables considered but not included in the final model: age, BMI, WHR, physical activity category, sex, fasting insulin quartile, fasting intact proinsulin quartile, and fasting 32,33 split proinsulin quartile.

the U.K. have used the point at which the disease is clinically recognized to define its onset (27,28). Harris (29) has previously reviewed studies reporting the average annual percentage deterioration to diabetes for subjects with IGT. These rates varied from 1.5% per annum in the Bedford Study (30) to 7.3% per annum in Mexican Americans in Colorado (31). However, in many instances, direct comparison is made difficult by differences in the study methods used (32,23). Other studies are not comparable because of differences in the population examined. Stengard et al. (33) reexamined glucose tolerance in survivors of the Finnish cohorts of the Seven-Countries Study who were aged 65–84 years at baseline. Of the 234 men who had IGT at baseline, a large proportion had either died or could not be reassessed at follow-up (37%). By contrast, 89% of the nondiabetic population from phase I of the Isle of Ely Study who were still alive at follow-up were reexamined in this study. Even though this response rate is high, there were differences between the subjects who were restudied and those that were unable or refused to help. Male volunteers were less likely to smoke and were on average slightly older than those who did not attend for rescreening. A small excess of women who were overweight declined to be reexamined. The association of nonresponse with smoking and obesity might be a typical "healthy participant" effect, with the direction of bias being toward the study of volunteers who had fewer conventional risk factors (34–36). In this particular study, the tendency toward recruitment of subjects with a lower risk of progression to diabetes was balanced by a high response rate in subjects with IGT at baseline. A higher recruitment of subjects with IGT would possibly bias the study toward overestimating the incidence of diabetes. Because the relative risk of IGT for diabetes is so high, the effect of a bias in this direction was likely to be the most serious. To estimate its effect, the incidence rate was recalculated to take account of loss to follow-up. However, the adjusted rate was very similar to the unadjusted rate, suggesting that even if this bias existed, its magnitude would be very small.

We conclude from this study that individuals with raised 32,33 split proinsulin and intact proinsulin, markers of β-cell dysfunction, are at increased risk of progression to diabetes. The measurement of the sum of these two proinsulin concentrations, combined with the fasting glucose and a family history of diabetes, can aid in the identification of a high-risk subgroup in whom the risk of progressing to diabetes at 4.5 years was 30%. The use of this approach in identifying a subgroup of individuals who may benefit from targeted intervention needs replication in other cohorts.

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