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The Interaction between Impaired Acute Insulin Response and Insulin Resistance Predicts Type 2 Diabetes and Impairment of Fasting Glucose

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Abstract

Background: Impaired acute insulin response (AIR) and insulin resistance (IR) are characteristics of Type 2 diabetes (T2DM). The aim was to develop risk models for T2DM and impaired fasting glucose (IFG), reflecting estimates both of AIR and IR, and of their interaction, as predictors over 20 years of follow-up.

Methods: We developed predictive models using hierarchic multiple regression analyses in a population-based cohort of 1227 men with normal fasting blood glucose at baseline (1970–73) and were reinvestigated after 10 and after 20 years. Using IVGTT-variables correlated either to AIR or to IR, separate models were developed. Combined models were also estimated from which prediction scores, representing individual risk, were calculated.

Results: In combined models, interaction between prediction scores reflecting AIR and IR predicted T2DM and IFG. Lowest tertile of AIR and the highest tertile of IR showed a relative risk (RR) of 15.3 (95%-CI=5.58–41.84) for T2DM compared to the contrast group (high AIR and low IR). Corresponding RR for IFG was 13.23 (95%-CI=6.53-26.78). C-statistic increased from 0.76 to 0.79 (p=0.018) for T2DM and from 0.77 to 0.80 for IFG (p=0.062) taking interaction into account. Main effects of lowest tertile of AIR and highest tertile of IR versus best were: RR for T2DM, 8.80 (95%-CI=4.25-18.21) and 6.31 (95%-CI=3.26-12.21); for IFG, 9.07, (95%-CI=5.38-15.29) and 4.49 (95%-CI=2.98-6-76).

Conclusion:. The interaction between low AIR and high IR revealed a high relative risk for T2DM or IFG reflecting the interplay between these factors over long time on worsening glucose tolerance and development of manifest disease.

Introduction

Insulin resistance (IR) reflected by high fasting plasma insulin concentrations, impaired acute insulin responsiveness (AIR) after a glucose load, obesity and high blood pressure have all been identified as independent predictors of Type 2 diabetes (T2DM) (1–5). Prediction models for T2DM, using sets of variables reflecting carbohydrate and lipid metabolism, anthropometric and hemodynamic variables, and/or data from questionnaires, have been developed previously in longitudinal studies ranging up to 8 years (6, 7).

Defects in insulin action and insulin secretion are the major abnormalities in the progression from normal fasting glucose and postprandial tolerance to impaired fast-

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ing glucose (IFG), impaired glucose tolerance and T2DM (3, 8). During each stage of the development of T2DM, IR and insulin secretory dysfunction are independent predictors of deterioration of glucose tolerance (9). Although a decreased AIR and IR are important characteristics predisposing to T2DM (8, 10, 11), the interaction between AIR and IR is less studied. Prediction models for T2DM based on long time of follow-up and using variables reflecting AIR, IR and their interaction have not been systematically validated. Further, the predictive capacities of variables associated with these characteristics and their interaction are largely unknown.

The primary aim of this study was twofold. First, to use variables derived from the IVGTT and reflecting AIR and IR, to study their interaction in relation to development of IFG and T2DM. Further, to develop a model – with high predictive capacity over a follow up period of 20 years – for T2DM and impaired fasting glucose (IFG) based upon fasting glucose concentrations using current diagnostic criteria (12). Secondly, we wished to calculate individual scores from the developed prediction models and to evaluate the predictive capacity of these models for the development of T2DM and IFG.

Research design and methods

Study population

The Uppsala Longitudinal Study of Adult Men (ULSAM) is a population-based study of diabetes and cardiovascular disease in men, the characteristics of whom have been described previously (13). All men born 1920 to 1924 and resident in the municipality of Uppsala, were invited to participate in a health survey between 1970 and 1973, hereafter called baseline. In all, 2322 out of 2841 men participated in the survey. The second survey, at age 60, was carried out between 1980 to 1984 and has been described elsewhere (4). At the third survey, carried out between 1991 and 1995, in all 1221 out of 1681 eligible men participated. The present study was based on those subjects (n=1227) who had normal fasting blood glucose (<5.6 mmol/l) at baseline, according to ADA criteria, as well as baseline data from an intravenous glucose tolerance test (IVGTT) and further fasting blood glucose concentrations measured at the second and/or at the third surveys. Subjects with pharmacological treatment for diabetes at baseline were excluded from this study.

The Ethics Committee of the Faculty of Medicine at the Uppsala University approved the study. Informed consent was obtained from all participants.

Glucose tolerance test

An IVGTT was performed on 1792 men at baseline (13). A glucose dose of 0.5 g/kg body weight was administered. Samples for glucose determinations were drawn before and at 20, 30, 40, 50 and 60 minutes after the start of the glucose injection. The serum insulin concentrations during the IVGTT were measured in duplicate

blood samples drawn before and 4, 6, 8 and 60 minutes after the start of the glucose injection. Fasting blood glucose concentrations were obtained in all participants at baseline and at the second survey and fasting plasma glucose concentrations were obtained in 1219 out of 1221 men at the third survey.

Biochemical determinations and BMI

At baseline and at the second survey blood glucose was analyzed by spectrophotometry using the glucose oxidase method. At the third survey plasma glucose was analyzed by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany).

The serum insulin concentration was determined with the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden).

Weight was measured to the nearest kg and height to the nearest cm. BMI was calculated as weight / height squared (kg/m^2) (13).

Statistics

Distribution of variables

Skewed variables were log transformed to reach normal distribution, w>0.95 determined with Shapiro Wilk's test (14). Normally distributed variables were used in all statistical analyses, which were performed using the following statistical software packages: SAS version 9.1 for Windows (SAS Institute Inc, NC, USA) and STATA 10.0 for PC (Stata Corporation, College Station, USA). All tests were two-tailed and a p-value < 0.05 was considered significant.

Definition of outcome variables

Following to the ADA criteria from 1997 (12), using fasting glucose concentrations only, as oral glucose tolerance tests (OGTT) were not performed at the first (1970–73) and second (1980–84) surveys, two separate outcome variables –the incidence from age 50 to age 70 for T2DM and IFG – were constructed. These dichotomous variables (diseased/non-diseased) are defined as: 1) T2DM [fasting glucose concentrations > 6.0 mmol/l (blood), >7.0 mmol/l (plasma)] and 2) IFG [fasting glucose concentrations > 5.6 mmol/l (blood), >6.0 mmol/l (plasma)].

Determination of an insulin index

Peak insulin was expressed as the mean of the serum insulin concentrations determined at 4 and 6 minutes. In order to control the peak insulin for the fasting insulin we constructed an insulin index reflecting AIR, which was defined as the ratio of the natural logarithm of the peak insulin minus 2.91 and the natural logarithm of the

fasting serum insulin concentration. The constant, 2.91, was the intercept in the linear regression of the natural logarithm of the peak insulin on the natural logarithm of fasting insulin. We subtracted the intercept from the nominator (peak insulin) to optimally reduce the influence of the denominator (fasting insulin) (15). This index was found to predict T2DM better than other measurements of first phase insulin release (AUC from 0 to 8 minutes or the mean insulin concentration, from 4, 6 and 8 minutes after an intravenous glucose load). It was less strongly associated with fasting insulin concentrations than the other measurements mentioned analysed with Pearson product moment correlations.

Choice of candidate variables

Variables from the IVGTT, as listed in Table 1 (with a correlation coefficient < -0.4 or > 0.4), that were more strongly correlated to the insulin index – than to fasting insulin as a surrogate marker for insulin resistance – were selected as candidate variables for models representing AIR. The variables, as listed in tab 1 (with a correlation coefficient < -0.4 or with a correlation coefficient > 0.4), that were more strongly correlated to fasting insulin than to insulin index were selected as candidate variables for models representing IR. Because the euglycaemic insulin clamp technique was not available at baseline (16), the surrogate marker fasting insulin, which correlates well with insulin sensitivity measured by the clamp technique, was used as a proxy for insulin resistance (17, 18).

Predictive models and relative risk measurements

The development of prediction models was performed using a hierarchic strategy of multivariable logistic regression models for the selection of candidate variables.

The selection of variables that were more strongly correlated to insulin index than to fasting insulin and that significantly predicted T2DM and IFG, including significant interactions constituted a model that best reflects AIR. Scores for acute insulin responsiveness (AIR-score) were calculated for each subject as the weighted sum of the parameter estimates of these significant predictors.

Using the same procedure, we developed a model representing IR performed on the variables that were more strongly correlated to fasting insulin than to insulin index and calculated individual scores (IR-score).

The AIR-score and the IR-score were then introduced, as independent variables, to a multiple logistic regression model and their interactions were tested for significance. These significant predictors constitute the best model representing AIR, IR and their interaction combined for the development of each of the two pre-defined outcomes. By dividing AIR and IR into tertiles, relative risk with 95% confidence intervals were calculated for contrast groups (lowest vs. highest).

Further, BMI as an explanatory variable was introduced into the combined models to study whether any of the significant relationships was altered.

To avoid confounding by age, final models were adjusted for age at baseline

Table 1. Clinical characteristics at baseline for subjects who developed Type 2 Diabetes Mellitus, Impaired Fasting Glucose or remained normoglycaemic, over the 20-year follow-up period (ADA 1997 criteria) and Pearson product correlation coefficients of variables from the intravenous glucose tolerance test with Insulin index and fasting insulin for the entire study population.

	Type 2 diabetes mellitus (n=78)	Impaired fasting glucose (n=88)	Normal glucose tolerance (n=1061)	Pearson product correlation coefficients (n=1643)	
	Mean ± SD	Mean ± SD	Mean ± SD	Insulin index	Fasting insulin
Fasting blood glucose (mmol/l)	5.0 ± 0.4 **	5.0 ± 0.4 **	4.8 ± 0.4	-0.20	0.22
Blood glucose at 60 min (mmol/l)	13.2 ± 2.9 ***	12.9 ± 2.9 ***	10.8 ± 2.7	-0.50	0.15
Fasting serum insulin (pmol/l)	105 ± 61 ***	95 ± 53 ***	73 ± 41	-0.0005	1.00
Serum insulin at 60 min (pmol/l)	268 ± 132 ***	237 ± 124 ***	161 ± 101	-0.10	0.52
Insulin index	0.38 ± 0.21***	0.38 ± 0.22 ***	0.51 ± 0.22	1.00	-0.0005
Peak insulin (pmol/l)	395 ± 261 n.s.	370 ± 241 *	440 ± 305	0.90	0.39
Body mass index (kg/m^2)	26.9 ± 3.5 ***	26.3 ± 3.4**	24.7 ± 2.9	_	_

Data are arithmetic means ± standard deviation presented for all variables. $p<0.001$ for all Pearson product correlation coefficients presented except that between Fasting serum insulin and Insulin index ($p=0.98$). *** denotes $p<0.001$, ** denotes $p<0.01$ and * denotes $p< 0.05$, normal glucose tolerance as the reference group. The entire study baseline population constituted of 1643 participants and outcome data presented for 1227 participants are for subjects who attended follow-up at reinvestigations after 10 and after 20 years.

(age was not a candidate variable in the regression model strategy), even though it was not significantly associated with outcome, due to a relatively short age span at baseline (mean 49.6 years, range 48.7–51.1 years).

Test of predictive capacities

The risk stratification of the individual patient was evaluated by the area under the Receiver-Operating-Characteristic curve (C-statistic). The increased discriminative value of the interaction term is expressed as the differences in C-statistics after the addition of the interaction-term between AIR and IR to a model with AIR and IR as explanatory variables and compared to a model including AIR and IR only. This was estimated for T2DM and IFG as the outcome in separate models and p-values were calculated.

The predictive capacities of the two final combined models, sensitivity and specificity, and percentage correctly classified were estimated using the jack-knife technique (19), which removes the overestimation-bias occurring when model estimation and outcome prediction are carried out on the same population of subjects. The incidences over 20 years for T2DM and IFG, were used as cut-off values in the jack-knife models.

Results

Incidence of T2DM during follow-up

During the course of the study 1061 subjects remained normoglycemic (86,4 %), 88 subjects developed IFG (7.2 %) and 78 subjects developed T2DM (6.4%).

Selection of candidate variables for predictive models

Table 1 presents baseline metabolic characteristics from the IVGTT for subjects whom later developed T2DM, IFG or remained normoglycemic. All candidate predictors of T2DM showed statistically significant differences between subjects who developed T2DM and those who remained normoglycemic. Results were essentially the same when IFG was defined as the outcome.

Table 1 also shows the association, given as correlation coefficients of insulin index and fasting insulin and variables from the IVGTT in the entire study population at baseline (n=1643). Peak insulin at 4 and 6 minutes and blood glucose at 60 minutes showed a stronger correlation to insulin index than to fasting insulin and was chosen together with serum insulin index, as candidate predictors reflecting AIR for calculation of the AIR-score. Serum insulin at 60 minutes showed a stronger correlation to fasting insulin than to insulin index. Therefore, insulin at 60 minutes was chosen, together with fasting serum insulin, as candidate predictor reflecting IR for calculation of the IR score.

Fasting blood glucose showed a correlation coefficient of -0.20 to serum AIR and 0.22 to fasting insulin and was therefore not chosen as a candidate variable in either of the models.

Predictive models

The significant predictors of T2DM, representing AIR, were: Insulin index, AIR and glucose at 60 minutes. Results for IFG were essentially the same but here the interaction between insulin index and AIR was also significant. The significant predictor of T2DM, as well as of IFG, representing IR was insulin at 60 minutes, whereas fasting insulin was not a significant predictor when insulin at 60 minutes was present in the model. Both the calculated score for AIR and the score for IR were statistically significant predictors of T2DM and IFG (Model equations are

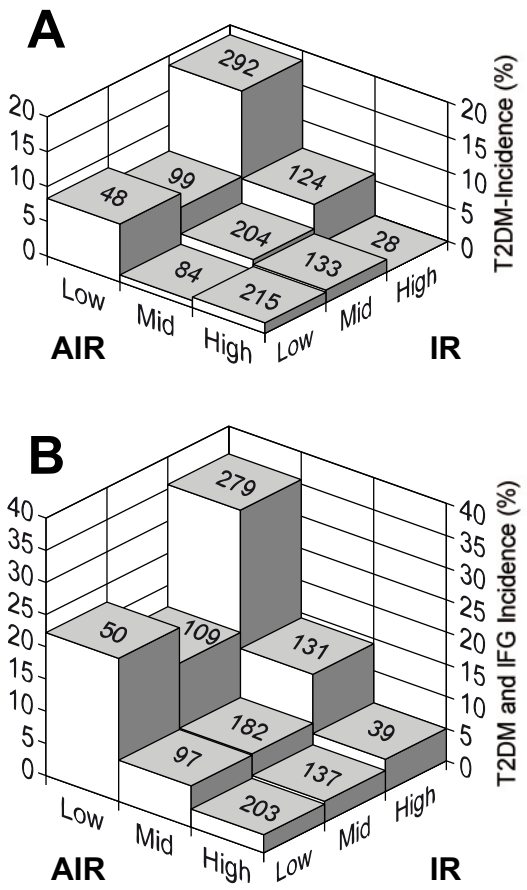

Figure 1 A-B – A: Progression from normal fasting glucose to Type 2 Diabetes (T2DM) and B: Progression from normal to impaired fasting glucose (IFG) over 20 years of follow-up from age 50, in a population based sample of 1227 men, as a function of the interaction between scores reflecting insulin resistance (IR) and acute insulin response (AIR).

The interaction between IR and AIR was significant when tested formally, p=0.004 (A) and p=0.008 (B). The actual number of subjects in each group, defined cross-sectionally by tertiles of AIR and IR, is given in the figure.

presented in the Appendix). Also, the interaction between the AIR-score and the IR-score was significant in both cases and was included in the combined models for predicting T2DM and IFG. Figures 1 A–B show the magnitude of the interaction between the scores, representing AIR and IR presented as tertiles, in subjects who developed T2DM (A) and in subjects who developed IFG and/or T2DMs (B). Adjustment for BMI did not alter any of the significant relationships in either of the two combined prediction models.

To get reliable estimates, the cut-off values for the tertiles of the AIR and IR scores were determined in the total study population (n=1643). When applied in Figure 1 the population is limited to subjects with complete data on both predictors and the outcome (n=1227). Therefore, the marginal distributions will differ from the expected 1/3 of the group in each tertile. The likely consequence is that our results underestimate rather than overestimate the true risk.

The interaction between AIR and IR scores for the development of IFG and T2DM

When comparing the group of subjects with ”low” AIR and ”high” IR to subjects in the contrast group with ”high” AIR and ”low” IR, a relative risk (RR) of 15.3 with a 95%-confidence interval (CI) of 5.58–41.84 for the development of T2DM was revealed. The corresponding RR for the development of IFG and T2DM was 13.23, CI=6.53-26.78. These relative risks, representing additive effects and the strong interaction effect, were higher than the following relative risks presented separately for AIR and IR. When comparing the lowest tertile, with the highest tertile of AIR, the RR was 8.80, CI=4.25-18.21 for the development of T2DM and 9.07, CI= 5.38-15.29 for the development of IFG or T2DM. Similarly, the relative risk, when comparing the highest tertile with the lowest tertile of IR, was 6.31, CI=3.26-12.21 for the development of T2DM and 4.49, CI= (2.98-6.76) for the development of IFG or T2DM.

Predictive capacities

The C-statistic increased from 0.76 to 0.79 (p=0.018) for T2DM and from 0.77 to 0.80 (p=0.062) for IFG when the interaction between AIR and IR was taken into account, respectively. The predicitve capacities of T2DM and of IFG of the two combined models including the calculated scores reflecting AIR and IR as well as the interaction term were: sensitivity 72/74%, specificity 78/74 and percentage correctly classified 78/74%.

We also evaluated the predictive capacity, for each condition, of a simplified model in which AIR and fasting insulin, as a surrogate marker for IR, constituted predictors. Percentage correctly classified was significantly lower, for each condition in these models (69% / 69%), $p<0.001$ (McNemars test for dependent proportions). Further, there was no significant interaction term in such simplified models.

Finally, an evaluation of the IR score was performed by using baseline data from the IVGTT and euglycemic insulin clamp (n=61) from a previous study (20). The IR score was calculated from the IVGTT data using the equation derived in ULSAM and analysed in relation to the gold standard measurement for insulin resistance, the insulin sensitivity index (M/I). The correlation coefficient between IR score and insulin sensitivity index (M/I) was high; 0.77 ($p<0.001$).

Discussion

In this large population based study, we have shown that scores reflecting acute insulin response (AIR) and insulin resistance (IR), both were significant predictors of the outcome, T2DM and IFG. Further, these AIR and IR scores were found to interact significantly already at 20 years before the clinical onset of disease, imply-

ing a multiplicative risk for the development of T2DM or IFG (Figures 1A–B). A smaller study of shorter duration up to five years, using both IVGTT and euglycemic insulin clamp, has shown that IR and insulin secretory dysfunction have independent and additive pathogenic roles during each stage of the development of T2DM (9). This and the present study are concordant with each other although there is a substantial difference in follow-up time. But more important our results reveal a significant interaction, which was not statistically significant in the smaller study, probably due to lack of power.

Our finding that the derived insulin index, reflecting impaired AIR in response to an intravenous glucose load adjusted for IR, is a significant factor predicting T2DM is in accordance with previous results obtained in Pima Indians (3) using the OGTT. Also, the finding, that impaired AIR significantly predicts IFG, is consistent with results obtained in studies with IGT as the outcome (21). The latter study showed that a latent defect in the AIR, expressed as change in insulin/glucose ratio over the first 30 minutes of an oral glucose tolerance test, predicted IGT better when adjusted for fasting insulin concentrations, similar to our calculation of an insulin index.

The present results, with special reference to AIR, are in good agreement with the notion that impaired insulin secretion is the initial and main genetic factor predisposing to T2DM (8). Even though both insulin secretory defects and IR are to a large extent genetically determined (8), increased insulin secretion compensates for IR and overt disease occurs gradually as beta cell compensation fails (10). Our results show that early signs of failing beta cell compensation precede disease onset by up to 20 years, although it seems to be interacting with and a concurrent phenomenon to IR. Thus, the combined predictive model takes into account the degree of IR when evaluating the AIR, as suggested by Kahn et al. (22). In this study population, we have not been able to compare the derived score for IR with measurements from a euglycaemic insulin clamp, as the technique was not available at our department at baseline in 1970–73 (16). However, as indicated in the Results section the IR score is highly correlated to the insulin sensitivity measured by the euglycemic insulin clamp using baseline data from a clinical trial (20). IR emerged as a significant predictor of T2DM in an earlier 25-year follow-up study of glucose-tolerant first-degree relatives of patients with T2DM (23), which may seem to be in contrast to our finding. However, the lack of selection bias in a population-based study (3) may reveal the significant effect of impaired AIR on the development of T2DM and IFG – as found here – while bias may occur when studying relatives of patients with T2DM (selection for IR) as in the referred study. Also, differences in age and gender of the study population may contribute to the different results. Furthermore, to optimize the AIR score by reducing the influence of IR variation within it when developing the insulin index, the significant effect of lower AIR on T2DM risk was revealed as was the interaction with IR.

There was also a contribution of the glucose values at 60 min of the IVGTT to the prediction score reflecting AIR. Plasma glucose at 120 min during an OGTT has also been shown to be a significant predictor of defects in beta cell function

(24). The difference in time points is likely to reflect the differences both in route of administration of glucose and in glucose absorption. Both values are, however, point estimates and represents overall integrated measurements of effect of both insulin secretion during the prolonged second phase as well as glucose clearance. We evaluated the prediction scores with the jackknife technique (19), which enables that prediction for each subject is made with this subject's data excluded and therefore the risk for over-fit is reduced. The predictive capacities of our models, measured in terms of percentage correctly classified were of a slightly lower magnitude (8% lower frequency of percentage correctly classified) than those generated by Stern et al. (6), which were based on an 8-year follow-up and evaluated by a cross-validation technique. The small differences in the predictive capacities are likely to depend on some loss of precision due to our longer follow-up time of 20 years (25). The interaction factors used in our combined models enhanced the predictive capacity. The better predictive capacity for T2DM alone than for IFG *and* T2DM combined is consistent with results (11, 26) from studies with shorter follow-up periods. Finally, our derived models predicted outcome significantly better (9%) than a more conventional model does, using AIR and fasting insulin as predictors due to more information introduced to the explanatory variables.

The prediction scores provide concise methods of summarizing those aspects of AIR and IR and their interaction that are predictive of T2DM or IFG, with a potential use to predict individuals who are at high risk to develop these conditions and who might be subjected to prevention activities (27). As an alternative to IVGTT, prediction scores from an OGTT could also be estimated and used as a test in high risk individuals. In a cross sectional setting, indices from an OGTT reflecting both AIR and IR have been derived in relation to gold standard methods (28) without, as yet, having been evaluated in longitudinal studies. Further, a prediction score can be used alone instead of a set of variables, for systematic testing of the contribution of any new genetic marker, for example single nucleotide polymorphisms identified in genome wide associations studies in T2DM, and it's possible relation to quantitative estimates of IR or AIR and it's value as a predictor of T2DM or IFG. In addition, the risk of developing the specified conditions may be calculated on an individual basis for all subjects with baseline data from the intravenous glucose tolerance test. Thus, in fact it may solve some of the problem of loss at follow-up that always occur in a longitudinal study, at least of longer duration.

In summary, the interaction between low AIR and high IR revealed a high relative risk for development of T2DM or IFG in the present study. The results indicate that AIR and IR have interactive pathogenic, concurrent roles during T2DM development over two decades. It suggests that detecting both AIR and IR should be regarded as targets in the prevention and treatment of T2DM (29, 30). Prediction models based on calculated scores representing AIR and IR combined, predict T2DM with high precision in this cohort. A shorter version using fasting insulin and AIR only, may be used to predict T2DM and IFG without much loss of predictive capacity. The scores can also be used, one at the time, when testing any new possible predictor, of T2DM systematically, e.g. gene polymorphisms.

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Appendix

Equations for the predictive models

Combined model for Type 2 diabetes mellitus.

(1) AIR score = –22.518 + (2.763 * lnPI) + (–8.447 * IX) + (0.225 * 1-hBG) + (0.196 *age)

(2) IRscore= –17.411 + (1.585 * ln1-hSI) + (0.189 * age)

(3) ln (P/1–P) = –4.379 + (9.518 * AIR score) + (13.289 * IR score) + (–32.644 * AIR score * IR score)

Combined model for impaired fasting glucose.

(4) AIR score= –12.594 + (2.914 * lnPI) + (–3.016 * IX) + (0.222 * 1-hBG) + (–1.278 *lnPI *IX) + (0.003 *age)

(5) IR score= –5.586 + (1.199 * ln1-hSI) + (0.005 * age)

(6) ln (P/1–P) = –4.171 + (9.241 * AIR score) + (8.157 * IR score) + (–18.104 * AIR score * IR score)

Abbreviations

AIR score = Acute Insulin Response Score, IR score = Insulin Resistance Score, ln = natural log, age = age (years), 1-hBG = Blood Glucose at 60 min (mmol/l), FSI = Fasting Serum Insulin (pmol/l), 1-hSI = Serum Insulin at 60 min (pmol/l), PI = Peak insulin of the acute insulin response (average serum insulin concentration at 4 and 6 min (pmol/l), IX = Insulin index, (P/1-P) = Probability that a diseased is sampled as a case divided by the probability that a healthy is sampled as a control.

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