Insulin resistance is a treatable precursor of diabetes and potentially of cardiovascular disease as well. To identify insulin-resistant patients, we developed decision rules from measurements of obesity, fasting glucose, insulin, lipids, and blood pressure and family history in 2,381 (2,138 nondiabetic) individuals studied with the euglycemic insulin clamp technique at 17 European sites; San Antonio, Texas; and the Pima Indian Reservation. The distribution of whole-body glucose disposal appeared to be bimodal, with an optimal insulin resistance cutoff of <28 μmol/min · kg lean body mass. Using recursive partitioning, we developed three types of classification tree models: the first, based on clinical measurements and all available laboratory determinations, had an area under the receiver operator characteristic curve (aROC) of 90.0% and generated a simple decision rule: diagnose insulin resistance if any of the following conditions are met: BMI > 28.9 kg/m², homeostasis model assessment of insulin resistance (HOMA-IR) > 4.65, or BMI > 27.5 kg/m² and HOMA-IR > 3.60. The fasting serum insulin concentrations corresponding to these HOMA-IR cut points were 20.7 and 16.3 μU/ml, respectively. This rule had a sensitivity and specificity of 84.9 and 78.7%, respectively. The second model, which included clinical measurements but no laboratory determinations, had an aROC of 85.0% and generated a decision rule that had a sensitivity and specificity of 78.7 and 79.6%, respectively. The third model, which included clinical measurements and lipid measurements but not insulin (and thus excluded HOMA-IR as well), had a similar aROC (85.1%), sensitivity (81.3%), and specificity (76.3%). Thus, insulin-resistant individuals can be identified using simple decision rules that can be tailored to specific needs. Diabetes 54:333–339, 2005
IDENTIFYING INSULIN-RESISTANT SUBJECTS

benefits of treating insulin-resistant nondiabetic individuals with insulin-sensitizing agents. Efforts to document the benefits of such treatment, however, have been hampered by the lack of an accepted method for assessing insulin resistance based on routine clinical measurements. Although a clinical trial could conceivably be performed based on enrolling insulin-resistant patients as defined by one of the definitive tests, translation of the results of such a trial into ordinary clinical practice would be problematic, given the lack of a clinical test for identifying the target population for treatment.

In the current study we have assembled what we believe to be the largest collection of euglycemic clamp data in the world from numerous research centers, and we have used recursively partitioned classification trees to develop decision rules for identifying insulin-resistant individuals based on routinely available clinical measurements.

RESEARCH DESIGN AND METHODS

The results of 2,321 (2,138 nondiabetic) euglycemic insulin clamp studies were assembled from several sources, including the European Group for the Study of Insulin Resistance (EGIR) project (n = 1,436), the Pima Indian Study (n = 597), and studies performed in San Antonio (n = 288, of whom 99 were Mexican American). The EGIR studies were performed on Caucasians from 17 European sites (Athens, Greece; Baden, Heidelberg, Kreisha, and Munich, Germany; Belgrade, Serbia; Geneva, Switzerland; Goteborg, Sweden; Helsinki and Kuopio, Finland; Odense, Denmark; and Naples, Padova, Pisa, Rome, Torino, and Verona, Italy). With the exception of the Pima sample, which was population-based, all of the other samples were recruited from clinical populations. The recruitment criteria and procedures and the euglycemic clamp protocols, which were similar at all sites, have all been described in original publications from these studies (14–16). In particular the same insulin infusion rate (40 mU/min m², equivalent to 1 mU/min kg body wt) was used in all studies. All procedures were approved by the institutional review boards of the institutions contributing data to this study, and all participants gave informed consent to the procedures.

The response variable was insulin-stimulated whole-body glucose disposal (µmol/min kg lean body mass). Predictor variables included sex, weight (kg), BMI (kg/m²), lean body mass (kg), waist and hip circumferences (cm), fasting glucose (mmol/l), fasting insulin (µmol/l), total cholesterol (mmol/l), LDL and HDL cholesterol levels (mmol/l), free fatty acids (µmol/l), triglycerides (mmol/l), systolic and diastolic blood pressure (mmHg), and family history of a first-degree relative with diabetes. We also evaluated certain combined variables, namely, the triglyceride-to-HDL ratio and the HOMA-IR (fasting insulin × fasting glucose)/22.5, with fasting insulin expressed in µU/ml and fasting glucose expressed in mmol/l (13).

Statistical methods

Bimodal normal mixture models. Based on the appearance of the histogram of the euglycemic clamp values, we elected to fit a bimodal normal mixture model to the distribution. Maximum likelihood methodology was used to estimate the means and standard deviations of the two hypothesized modes (17,18). In addition, the suitability of the bimodal normal mixture model was assessed using likelihood ratio testing and graphic diagnostics. The optimal cut point for distinguishing insulin-resistant from insulin-sensitive individuals based on clamp measurements was then set so as to maximize the sum of theoretical sensitivity and specificity, as determined from the fitted bimodal normal mixture distribution. The distribution of clamp values among diabetic subjects was used to further assess the suitability of the cut point suggested by the bimodality analysis.

Classification trees. Recursively partitioned classification trees were used to model the relationship between insulin resistance and various combinations of clinical covariates. Sequential partitioning or “splitting” of the covariate space produces an ever-expanding number of “compartments” or “nodes.” Each successive split is chosen to minimize the associated binomial deviance, so that nodes become increasingly homogeneous with respect to the proportion of individuals within them who are either insulin resistant or insulin sensitive (19,20). In theory this process can continue until all nodes are completely homogeneous, although this would result in an inordinately large number of nodes. Moreover, such an approach would inevitably lead to overfitting the data at the expense of generalizability to other datasets. Using cross-validation, however, it is possible to appropriately assess the balance between the discrimination of the two classes of individuals and the risk of overfitting the trees (20). A random 10-fold cross-validation was performed by dividing the dataset into 10 random subsets and then using all possible combinations of 9 subsets (development subsets) to develop sequences of trees of different sizes that were then tested on the remaining 10th subset (validation subset). The results of the cross-validation provide an unbiased assessment of the predictive accuracy of the tree models. We used “cost-complexity pruning” (19) to identify the tree size that optimized the trade-off between two competing aims: internal discrimination and external validity. Cost-complexity pruning measures the adequacy of any given tree model using a penalized version of its degree of node homogeneity (as measured by the binomial deviance), where the penalty is proportional to the size of the tree, i.e., its number of nodes. In addition, the random 10-fold cross-validation results allow for investigation of the consistency of split choices in the 10 development subsets, as well as the sensitivities, specificities, and areas under the receiver operating characteristic curves (aROCs) on each of the validation subsets.

Subjects for whom certain covariate data were missing were retained in the analyses using the method of “surrogate splits” (21,22). This method assigns a secondary, “surrogate” covariate to each split in the tree model, allowing classification of individuals with missing values for the primary covariate to be made on the basis of the associated surrogate covariate. The choice of surrogate covariates is made by identifying the covariate split that most closely matches the actual split among those individuals for whom both the actual and the surrogate covariates are available. There were no missing values for BMI. For the other covariates that figured in the ultimate decision rules, namely HOMA-IR, family history, and triglycerides, the percentages of missing values were 6.1, 28.1, and 34.8%, respectively.

Covariate selection. Initially, a screening tree was fit using all available predictors except ethnicity and age. Exclusion of ethnicity allowed construction of models for insulin resistance based on physiological and biochemical covariates with which ethnicity may have been associated. Such models may be more generalizable, particularly to ethnic groups not represented in the dataset. We also did not use age in the modeling process because age is minimally if at all, related to insulin resistance (14). Moreover, in the current dataset, Pima Indians tended to be younger than the other ethnic groups; none of the Pima Indians, for example, was aged >50 years, whereas 21.8% of San Antonio subjects and 31.8% of EGIR subjects were aged >50 years. Thus, age tended to act as a surrogate marker for Pima Indians. The covariates that significantly contributed to the screening tree were used as a covariate subset for further, more focused model fitting. Because of the recursive, binary splitting structure of tree model construction, there is no need to explicitly include covariate interactions or transformations.

In addition to the tree model incorporating all available covariates, two additional tree models were fit based on predictor subsets chosen to reflect various practical considerations, such as the ease of obtaining and the degree of sensitivity of the covariate measurements. The first of these two additional models was based on routine clinical measurements, excluding any that required obtaining a blood specimen. The second model was fit using these same clinical measurements, but it also incorporated the lipid measurements, but not the insulin measurement.

Development of decision rules. Once subjects have been assigned to nodes, a decision rule for classifying them can be developed by labeling certain of the nodes as “test positive” (i.e., insulin resistant, in the current instance) and the remaining nodes as “test negative.” The choice of which nodes to label as “test positive” is based on the proportion of “true positive” individuals (i.e., insulin resistant by clamp) in that node. The proportions of true positives in each terminal node can be thought of as an insulin resistance “score” for that node, and the decision as to which scores correspond to “test positive” or “test negative” can be chosen with a view to balancing sensitivity and specificity considerations, depending on program needs.

RESULTS

Optimal cutoff for determination of insulin resistance. Figure 1 presents histograms of the insulin clamp measurements for all 2,321 subjects and for the 2,138 nondiabetic subjects. The histogram excluding diabetic subjects is scaled to an area of 2,138/2,321, the observed prevalence of nondiabetic subjects in the data, allowing more direct comparison with the histogram including diabetic subjects. Both histograms are compatible with bimodality, and it appears that the lower mode, associated with diabetic subjects, contains a greater proportion of subjects than the upper mode, associated with nondiabetic subjects. Statistical tests suggest that the histograms are compatible with a bimodal normal mixture model, with a higher mean for diabetes and a higher variance for nondiabetes.

Figure 2A presents a box plot of distribution of insulin clamp values across tertiles of BMI. Statistically significant differences in insulin clamp values are seen between tertiles of BMI, with the lowest tertile (BMI ≤ 24.8 kg/m²) showing lower clamp values than the two higher tertiles of BMI. Figure 2B presents data from a two-way analysis of variance (ANOVA) of log-transformed insulin clamp values across tertiles of BMI and tertiles of triglyceride levels, with a significant interaction between BMI and triglyceride levels, indicating that the effect of BMI on insulin clamp values is modified by triglyceride levels. Figure 2C presents similar data to Figure 2B, but includes tertiles of HDL cholesterol levels instead of triglyceride levels. Again, a significant interaction between BMI and HDL cholesterol levels is seen. These data suggest that BMI and lipid levels are independently associated with insulin clamp values and that the effect of BMI on insulin clamp values is modified by triglyceride and HDL cholesterol levels.

Figure 3 presents a box plot of distribution of insulin clamp values across tertiles of BMI for different levels of HDL cholesterol levels. Statistically significant differences in insulin clamp values are seen between tertiles of BMI, with the lowest tertile (BMI ≤ 24.8 kg/m²) showing lower clamp values than the two higher tertiles of BMI. Figure 3B presents similar data to Figure 2B, but includes tertiles of HDL cholesterol levels instead of triglyceride levels. Again, a significant interaction between BMI and HDL cholesterol levels is seen. These data suggest that BMI and HDL cholesterol levels are independently associated with insulin clamp values and that the effect of BMI on insulin clamp values is modified by HDL cholesterol levels.
The estimated bimodal normal mixture density is overlaid on the plot. A likelihood ratio test comparing the bimodal mixture model to a single distribution model confirmed that the former was preferred \((P < 0.0001)\). The maximum likelihood estimates for the model parameters are shown in Table 1. Also shown in Table 1 for comparison are the mean and standard deviation for the clamp values in the diabetic subjects. Because Pima Indians constituted a sizeable proportion of our dataset \((597/2,138 = 27.9\%)\), and because Pima Indians are well known to be very insulin resistant, we checked for bimodality after excluding this group. Again, a bimodal mixture model fit the data significantly better than a single distribution model \((P < 0.0001)\).

In view of the evidence for bimodality in the distribution of clamp measurements, we used this information to assist us in picking a cut point to define insulin resistance, rather than select a purely arbitrary cut point. The “theoretical” prevalence of insulin resistance in the nondiabetic population, as estimated from the bimodal mixture model, was 23.1\%, which is in line with previously published estimates \((3)\). The optimal cut point for identifying nondiabetic insulin-resistant individuals, based on maximizing the sum of theoretical sensitivity and specificity, as determined from the fitted bimodal normal mixture distribution, was 28 \(\mu \text{mol/min} \cdot \text{kg lean body mass}\). This cut point gave an estimated sensitivity and specificity of 97.3 and 85.6\%, respectively, relative to the theoretical prevalence from the bimodal mixture model, and 32.7\% of the subjects fell below the cut point, i.e., were considered to be insulin resistant. Using this same cut point, it was found that 92.9\% of diabetic subjects were insulin resistant. Thus, the cut point based on the bimodality analysis closely approximates a cut point based on the 95th percentile of the distribution of glucose disposal rates in diabetic subjects.

### Table 1
Maximum likelihood estimates for the bimodal mixture model fit to insulin clamp values

<table>
<thead>
<tr>
<th>Group</th>
<th>Means ± SD ((\mu \text{mol/min} \cdot \text{kg lean body mass}))</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistant</td>
<td>19.1 ± 4.6</td>
<td>0.23</td>
</tr>
<tr>
<td>Non–insulin resistant</td>
<td>46.0 ± 16.9</td>
<td>0.77</td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>17.1 ± 8.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

Tree models for predicting insulin resistance using all predictors. In the tree model based on all of the predictors, the following predictors were statistically significant: HOMA-IR, fasting plasma insulin, BMI, waist circumference, and LDL cholesterol. Thus, subsequent, more-focused tree model analysis considered only these variables. HOMA-IR was included in the final model rather than fasting plasma insulin because, in the tree model construction process, it tended to be selected ahead of the latter by the recursive partitioning algorithm. However, because HOMA-IR and fasting plasma insulin were highly correlated \((r^2 = 0.969)\), the fasting plasma insulin cut points corresponding to the HOMA-IR cut points can be calculated from the regression equation: fasting plasma insulin \((\text{in pmol/l}) = 6.786 + (25.314 \times \text{HOMA-IR})\).

Figure 2 depicts the classification tree model using the predictors HOMA-IR, BMI, waist circumference, and LDL. The aROC for this model is 90.0\%. The number of insulin-resistant individuals and the total number of individuals (insulin resistant + non–insulin resistant) in each node are presented in Fig. 2. The proportion of insulin-resistant individuals is shown for each of the eight terminal nodes (labeled 1 through 8).
If we declare nodes in which the proportion of insulin-resistant individuals is \( \geq 0.25 \), i.e., nodes 4–8 in Fig. 2, to be test positive, then the associated decision rule takes the simple form of predicting an individual to be insulin resistant if any of the following conditions are met:

1) HOMA-IR > 4.65
2) BMI > 28.9 kg/m\(^2\)
3) HOMA-IR > 3.60 and BMI > 27.5 kg/m\(^2\)

The insulin concentrations corresponding to the HOMA-IR cutoffs of 4.65 and 3.60 are 124.5 pmol/l (20.7 U/ml) and 97.9 pmol/l (16.3 U/ml), respectively. The insulin concentrations corresponding to the other HOMA-IR cutoffs are given in the legend to Fig. 2.

The above prediction rule has an estimated sensitivity and specificity of 84.9 and 78.7%, respectively, obtained by summing the insulin-resistant and the non–insulin-resistant individuals in the nodes declared to be test positive (nodes 4–8, true positives and false positives) and similarly obtaining the true negatives and the false negatives from the remaining nodes (nodes 1–3). Other choices for the predictive cutoff value (i.e., other than 0.25) will lead to different prediction rules and different associated sensitivities and specificities. The results of the random 10-fold cross validation showed a strong degree of consistency in the splitting choices for the 10 development subsets and also satisfactory external validity (as judged by the sensitivities, specificities, and aROCs at the 0.25 prediction cutoff) in the 10 validation subsets (online appendix [available at http://diabetes.diabetesjournals.org]).

Models using clinical variables plus lipid measurements. Figure 4 shows the fitted tree based on the clinical predictors plus lipid measurements. The aROC for this tree was 85.1%, virtually identical to the tree that did not include the lipid variables (aROC of 85.0%). At the 0.25 prediction cutoff level (nodes 3 and 5–8), the decision rule for insulin resistance is to predict an individual to be insulin resistant if any of the following conditions are met:

1) BMI > 28.7 kg/m\(^2\)
2) BMI > 27.0 kg/m\(^2\) and family history of diabetes is positive, or
3) family history of diabetes is negative, but triglycerides > 2.44 mmol/l. This decision rule has an estimated sensitivity and specificity of 81.3 and 76.3%, respectively.

DISCUSSION

The current results suggest that the distribution of whole-body glucose disposal rates is bimodal. The presence of bimodality facilitates the choice of a cut point for defining insulin resistance that has some basis in the underlying biology and is not wholly arbitrary. It must be acknowledged, however, that unspecified population differences could have contributed to the appearance of bimodality in the glucose disposal rate distribution. In particular, although the protocols for the euglycemic clamp studies were nominally similar at all sites, methodological differences between the sites could have affected the distribution. Thus, if glucose disposal rates had been systematically underestimated relative to some “true” value in highly insulin-resistant individuals (e.g., Pima Indians) and/or overestimated in more insulin-sensitive
individuals (e.g., Caucasians) relative to the same gold standard, such a methodological bias would have tended to drive the Caucasian and the Pima means apart and perhaps have generated the appearance of bimodality. Although there is no evidence that such a bias was operating, we cannot definitively exclude it. We are re-

FIG. 3. Tree model based on clinical covariates only, i.e., not requiring blood specimens. FamHx, family history; IR, insulin resistant; N, no; NIR, non–insulin resistant; pIR, proportion with insulin resistance; Y, yes.

FIG. 4. Tree model based on clinical covariates plus lipids. DBP, diastolic BP; FamHx, family history; IR, insulin resistant; N, no; NIR, non–insulin resistant; pIR, proportion with insulin resistance; TG, triglycerides; Y, yes.
assured, however, that the cut point we have chosen based on the bimodality analysis is reasonable because it corresponds to a cut point generated by an independent method. Specifically, the bimodality-driven cut point closely approximates the 95th percentile of the distribution of glucose disposal rates observed in diabetic subjects. Thus, the normal glucose-tolerant individuals who were defined as being insulin resistant had glucose disposal rates corresponding to the bottom 92.9% of clamp values obtained in the individuals with diabetes, a condition known to be associated with moderate to severe insulin resistance (23). Interestingly, a trimodal distribution of maximal insulin-stimulated glucose uptake rates has been reported in Pima Indians and has been interpreted as evidence for a single gene effect with a codominant mode of inheritance (24). Unfortunately, these results cannot be directly compared with the current results because the earlier Pima analyses were based on maximal insulin-stimulated glucose uptake that was achieved using a 10-fold higher insulin infusion rate than that used in the studies included in the current analyses.

Using recursively partitioned classification trees, we have developed simple decision rules for identifying individuals deemed insulin resistant by the euglycemic insulin clamp technique. These decision rules are based on routine clinical measurements and appear to have acceptable sensitivity and specificity. Also, by performing a random 10-fold cross validation and by focusing on physiological and biochemical variables, rather than study-specific variables such as ethnicity, we hoped to enhance the ultimate generalizability of the decision rules. Nevertheless, it must be acknowledged that certain populations, such as Asians, may be more insulin resistant for a given BMI than Caucasians (25,26). Thus, the performance of our decision rules may be suboptimal in these populations.

The most accurate decision rule is based on HOMA-IR (which requires a measurement of fasting insulin concentration) and BMI. However, only slightly less accurate rules can be derived that do not require insulin measurements or that do not require obtaining a blood specimen at all. In view of the lack of standardization of insulin assays, these latter rules may be preferred.

The sensitivities and specificities of the decision rules we have presented flow from our decision to declare nodes to be test positive if the proportion of insulin-resistant individuals in them was $\geq 0.25$. Applying other thresholds to our classification trees leads to different decision rules with different sensitivities and specificities. If, for example, one desires a highly specific rule, one might choose to declare as test positive only individuals in nodes in which the proportion of truly insulin-resistant individuals (by clamp) was at least 40% (nodes 5–8 in the classification tree depicted in Fig. 2). This would lead to the following decision rule: declare an individual to be test positive if HOMA-IR $>4.65$ or if HOMA-IR $>3.60$ and BMI $>27.5$ kg/m$^2$; otherwise, declare that individual to be test negative. This rule has a sensitivity and specificity of 77.0 and 88.4%, respectively. Alternatively, if one accepted as insulin resistant those in nodes where the proportion of individuals with the condition was 50% or better (nodes 6–8 in the classification tree depicted in Fig. 2), the decision rule would be to declare the individual to be test positive if HOMA-IR $>4.65$; otherwise, declare the individual to be test negative. This rule has a sensitivity and specificity of 71.4 and 92.0%, respectively.

Highly specific decision rules, such as those just discussed, might be useful for a clinical trial where one wished to be highly certain that the enrolled participant actually had the condition in question and where sensitivity was a lesser concern. Of course, such a strategy might mean that when the results of the clinical trial, assuming a positive outcome, were translated into clinical practice, the public health impact might be compromised because, owing to the reduced sensitivity of the entry criteria, many who might have benefited from the treatment would not have been deemed eligible for the trial.

Classification trees are a nonparametric alternative to classical statistical discrimination techniques, such as logistic regression. Their advantages include the lack of a required a priori choice of model structure for the predictors and interactions, the ease of incorporation of observations with missing covariate values, and the simplicity and interpretability of the resultant prediction rules. Because the partitioning scheme is recursive, each successive split of the predictor space is conditional on all previous splits. Thus, interactions or nonlinear structures in the relationship between the predictors and the response variable can be captured automatically. For example, if a split occurs based on a particular cut point for BMI, it may be that the subsequent split for those above the BMI cut point might be based on HOMA-IR, whereas the subsequent split for those below the BMI cut point might be based on triglycerides. Such a three-way interaction term would almost never be detected by traditional regression techniques such as multiple logistic regression analysis, where it would rarely, if ever, be sought in the absence of a powerful prior hypothesis. In addition, because the splits are simple bifurcations along predictor axes, the process is invariant to monotonic rescalings of the predictors. Thus, whether a predictor or, say, its logarithm is used has no effect on the resultant analysis.

In the current instance, multiple logistic regression analyses were also performed and gave results, in terms of sensitivities, specificities, and aROCs, that were generally similar to those obtained with the tree-based models (27). Application of logistic regression models, however, requires computing scores that are typically less readily interpretable than decision rules based on tree models. Moreover, the structure of a decision tree often leads to insights into the data that are not as easily gleaned from classic parametric analyses without a more detailed mathematical understanding of their model structure. In addition, decision trees permit some individuals to be classified on the basis of only one, or at most a few, measurements, whereas scores derived from multiple logistic regression models require that all covariates be available.

Tree-based models typically make use of a greater percentage of the available data. Logistic regression models, on the other hand, are limited to individuals for whom none of the covariates are missing, unless one imputes values for missing covariates. This practice is often discouraged, however. A disadvantage of imputed values is that they are not "real" data, whereas surrogate splits, used
to maximize the amount of the data being utilized, are based on genuine observations.

Recently, McLaughlin et al. (28) reported that among 258 overweight individuals, fasting insulin, triglycerides, and the triglyceride-to-HDL ratio were the best predictors of insulin resistance, as defined by the insulin suppression test. The sensitivities of their cutoff points ranged from 57 to 67% and the specificities from 68 to 85%. These results are not necessarily incompatible with our results because they pertain specifically to overweight individuals (≥25 kg/m²), among whom the effect of BMI itself would presumably be attenuated, permitting the emergence of other predictors, in this case triglycerides and the triglyceride-to-HDL ratio. Moreover, it should be noted that our other predictors, in this case triglycerides and the triglyceride-to-HDL ratio, presumably be attenuated, permitting the emergence of insulin-resistant (i.e., diabetes and cardiovascular disease) will be incorporated into clinical trials and ordinary clinical practice.

In conclusion, we have shown that it is possible to identify individuals who are insulin resistant using routine clinical measures, thereby improving the likelihood that recognition of this important harbinger of serious diseases (i.e., diabetes and cardiovascular disease) will be incorporated into clinical trials and ordinary clinical practice.

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