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Insulin Resistance Probability Scores for Apparently Healthy Individuals

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Context: Insulin resistance (IR) can progress to type 2 diabetes. Therefore, timely identification of IR could facilitate disease prevention efforts. However, direct measurement of IR is not feasible in a clinical setting.

Objective: Develop a clinically practical probability score to assess IR in apparently healthy individuals based on levels of insulin, C-peptide, and other risk factors.

Design: Cross-sectional study.

Participants: Apparently healthy individuals who volunteered to participate in studies of IR.

Main Outcome Measure: IR, defined as the top tertile of steady-state plasma glucose during an insulin-suppression test.

Results: In a study of 535 participants, insulin, C-peptide, creatinine, body mass index (BMI), and triglycerides to high-density lipoprotein cholesterol ratio (TG/HDL-C) were independently associated with IR (all $P < 0.05$) in a model that included age, sex, ethnicity, BMI, blood pressure, insulin, C-peptide, fasting glucose, low-density lipoprotein cholesterol, TG/HDL-C, alanine aminotransferase, and creatinine. For an IR probability score based on a model that included insulin, C-peptide, creatinine, TG/HDL-C, and BMI, the odds ratio was 26.7 (95% CI 14.0 to 50.8) for those with scores >66% compared with those with scores <33%. When only insulin and C-peptide were included in the model, the odds ratio was 15.6 (95% CI 7.5 to 32.4) for those with scores >66% compared with those with scores <33%.

Conclusions: An IR probability score based on insulin, C-peptide, creatinine, TG/HDL-C, and BMI or a score based on only insulin and C-peptide may help assess IR in apparently healthy individuals.

Freeform/Key Words: insulin, insulin resistance, C-peptide

The rate of insulin-mediated glucose disposal varies several-fold in an apparently healthy population [1]. Those with the least effective insulin-mediated glucose disposal are considered to have insulin resistance (IR), which is associated with clinical syndromes such as cardiovascular disease, type 2 diabetes, hypertension, stroke, nonalcoholic fatty liver disease, polycystic ovary disease, and certain forms of cancer [2–8]. Apparently healthy individuals

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; IR, insulin resistance; SBP, systolic blood pressure; SSPG, steady-state plasma glucose; TG, triglyceride; TG/HDL-C, triglycerides to high-density lipoprotein cholesterol ratio.

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who are insulin resistant may benefit from interventions intended to enhance insulin sensitivity, thereby reducing the risk of these clinical syndromes.

IR can be assessed by estimating insulin-mediated glucose disposal using a hyperinsulinemic-euglycemic glucose clamp [9] or insulin-suppression test [10]. Both of these methods assess insulin-mediated glucose disposal under steady-state conditions. However, because these methods are time consuming, labor intensive, and require intravenous infusion, they are impractical in a primary prevention clinic.

Fasting insulin levels are correlated with insulin-mediated glucose disposal and can be readily measured in serum. Also, a number of calculated indirect assessments of IR [*e.g.*, homeostasis model assessment of IR (HOMA-IR)] [11] are based in part on fasting insulin levels. Unfortunately, insulin levels are typically assessed by immunoassays that differ in their capture and detection antibodies. Thus, these insulin immunoassays can produce a range of values for the same samples [12]. Recently, a validated high-throughput, mass-based, multiplex assay for both insulin and C-peptide has been reported, and this assay has been standardized to World Health Organization reference materials [13, 14]. In this study, our goal was to develop a clinically practical method to assess IR in apparently healthy individuals using IR probability scores, scores that are based on mass-based measures of insulin and C-peptide levels as well as established risk factors.

1. Subjects and Methods

A. Study Population

This is a cross-sectional analysis of a study in which participants (n = 1072) were recruited from the San Francisco Bay area through advertisements in the local newspapers and had volunteered to participate in studies of IR between 1999 and 2011. Recruitment was open to all those who responded to public advertisements and met inclusion and exclusion criteria. The study excluded pregnant women, those older than 80 or younger than 18 years, those with a history of cardiovascular disease, and those treated with insulin. All study participants were apparently healthy. For the current analysis, we further excluded those with fasting glucose (FG) ≥126 mg/dL (6.99 mmol/L) or those taking glucose-lowering medications (n = 149) and those with any missing data (n = 388). The remaining 535 participants were included in the current analysis. Each of the study protocols was approved by Stanford University's Institutional Review Board, and all participants gave written informed consent to participate in the studies.

B. Data Collection

Race and ethnicity were self-reported. Weight and height were measured while participants were wearing light clothing without shoes. Body mass index (BMI) was calculated by dividing weight in kilograms by squared height in meters. Blood pressure was measured using an automatic blood pressure recorder after participants were seated quietly in a chair for 5 minutes with their feet on the floor and their arm supported at heart level. The average of three blood pressure readings taken at 1-minute intervals using an appropriately sized cuff was recorded. Metabolic syndrome was defined as the presence of three or more of the following five characteristics: (1) BMI $> 30\ \mathrm{kg \cdot m^{-2}}$, (2) FG ≥ 100 mg/dL (5.55 mmol/L), (3) hypertension, defined as systolic blood pressure (SBP) ≥130 mm Hg or diastolic blood pressure (DBP) ≥85 mm Hg, (4) low high-density lipoprotein cholesterol (HDL-C), defined as <50 mg/dL (1.29 mmol/L) in women and <40 mg/dL (1.04 mmol/L) in men, and (5) triglycerides (TGs) ≥150 mg/dL (1.69 mmol/L).

After an overnight fast, subjects were admitted to the Stanford General Clinical Research Center for metabolic tests that included an insulin-suppression test as described later [10]. Concentrations of lipid and lipoproteins, creatinine, and alanine aminotransferase (ALT) were determined in fasting plasma samples by standardized methods at the clinical laboratory of Stanford University Medical Center. Plasma glucose concentrations were measured

on a Beckman glucose analyzer (Beckman Coulter). Serum samples used for the measurement of insulin and C-peptide were aliquots from fasting baseline samples of the insulin-suppression test protocol [10]. Measurement of insulin and C-peptide were performed as previously described [13] at Quest Diagnostics Nichols Institute.

To assess IR, insulin-mediated glucose disposal was quantified using the modified version of the insulin-suppression test [10]. Specifically, an intravenous catheter was placed in each arm. One catheter was used to draw blood samples and the other to administer a 180-minute infusion of octreotide (0.27 $\mu g \cdot m^{-2} \cdot min^{-1}$), insulin (32 $mU \cdot m^{-2} \cdot min^{-1}$), and glucose [267 mg (14.82 mmol)/$L \cdot m^{-2} \cdot min^{-1}$]. Blood was sampled at 150, 160, 170, and 180 minutes after the start of infusion to determine the steady-state plasma glucose (SSPG) and steady-state plasma insulin concentrations. During the insulin-suppression test, octreotide suppresses endogenous insulin secretion and similar hyperinsulinemic steady-state plasma insulin concentrations are achieved in all individuals. The magnitude of the SSPG concentration thus provides a direct measure of the ability of insulin to mediate disposal of the infused glucose load; that is, the higher the SSPG concentration, the more insulin resistant the individual. Insulin-mediated glucose disposal quantified by the insulin-suppression test highly correlates with that obtained by the hyperinsulinemic-euglycemic glucose clamp [15]. In this analysis, individuals were defined as having IR if their SSPG concentration was in the top tertile [≥198 mg/dL (10.99 mmol/L)] of SSPG of our study [16].

C. Statistical Methods

Differences in the biochemical and anthropometric characteristics between those with and without IR were assessed by Wilcoxon rank-sum test or *t* test for continuous variables and by χ^2 test for discrete variables. The association of study variables with IR was assessed in logistic regression models that adjusted for the covariates indicated in the tables. Because SSPG histograms appeared different for non-Hispanic whites (n = 335), Hispanics (n = 42), and others (n = 158), ethnicity was coded as a categorical variable for these three groups. The relationship between insulin and C-peptide was assessed by Spearman correlation. The tertiles of insulin and C-peptide were determined using empirical cumulative distribution function. Score components were selected in a stepwise regression. All variables were eligible to enter the model. First, the most noteworthy variable was added to the model. Then the remaining variables were assessed individually, and the most prevalent variable was added if its significance level was below a specified inclusion level. The variables now in the model were assessed, and the least noteworthy variable was removed if its significance was increased above a specified retention level. This process was repeated until no more variables could be added or removed from the model using the inclusion and retention significance levels indicated in the text. Score coefficients were estimated in models that adjusted for all study variables not included in the score; for example, coefficients for the score based on insulin and C-peptide were estimated in a model that included the score, age, sex, ethnicity, creatinine, BMI, TGs to HDL-C ratio (TG/HDL-C), FG, SBP, DBP, low-density lipoprotein cholesterol (LDL-C), and ALT. All *P* values are two-sided. All analyses were performed using R [17] or SAS version 9.2.

2. Results

The characteristics of the study participants are shown in Supplemental Table 1 according to IR status. Insulin-resistant participants had higher levels of FG, insulin, C-peptide, TG, TG/HDL-C, ALT, BMI, and SBP. Insulin-resistant participants had lower levels of HDL-C and LDL-C.

We investigated the association between study variables and IR in a model that included age, sex, ethnicity, FG, insulin, C-peptide, LDL-C, TG/HDL-C, creatinine, ALT, BMI, as well as SBP and DBP (Table 1). In this fully adjusted analysis, insulin, C-peptide, creatinine, BMI, and TG/HDL-C were associated with IR. The odds of being insulin resistant [SSPG in the top tertile, ≥198 mg/dL (10.99 mmol/L)] were 1.2-fold higher (95% CI 1.1 to 1.4) for each 10-pmol/L

Table 1. Association of Biochemical and Anthropometric Measures With IR

	Age, Sex, Ethnicity Adjusted			Fully Adjusted[a]		
	OR	**95% CI**	***P* Value**	**OR**	**95% CI**	***P* Value**
Age	0.9	0.7–1.1	0.2	0.9	0.7–1.2	0.5
Male sex	0.8	0.6–1.3	0.4	0.6	0.3–1.2	0.1
FG	1.8	1.5–2.2	1×10^{-8}	1.1	0.9–1.4	0.4
Insulin	1.6	1.5–1.8	4×10^{-25}	1.2	1.1–1.4	6×10^{-4}
C-peptide	2.2	1.9–2.6	3×10^{-26}	1.6	1.3–2.0	1×10^{-5}
TG/HDL-C	1.9	1.5–2.5	3×10^{-8}	1.4	1.0–1.8	0.03
LDL-C	0.8	0.7–1.0	0.05	0.9	0.7–1.2	0.4
Creatinine	0.9	0.7–1.2	0.6	0.7	0.5–0.9	0.01
ALT	1.4	1.1–1.7	0.003	1.0	0.7–1.3	0.9
BMI	3.0	2.3–3.9	4×10^{-16}	1.5	1.1–2.0	0.02
SBP	1.4	1.2–1.8	2×10^{-4}	1.1	0.7–1.5	0.8
DBP	1.2	1.0–1.5	0.03	1.0	0.7–1.4	0.9
Ethnicity			0.03			0.5
Hispanic	2.5	1.3–4.7	0.007	1.3	0.6–3.1	0.5
Other	1.2	0.8–1.8	0.5	1.4	0.8–2.5	0.2
Non-Hispanic white	1.0	Reference		1.0	Reference	

Odds ratios (OR) are per 1 SD, except for insulin (per 10 pmol/L) and C-peptide (per 100 pmol/L).
[a]From a model that includes age, sex, ethnicity (Hispanic, non-Hispanic white, or other), FG, insulin, C-peptide, LDL-C, TG/HDL-C, creatinine, ALT, BMI, SBP, and DBP.

increase in insulin, regardless of the level of C-peptide and other study variables. Similarly, the odds of being insulin resistant were 1.6-fold higher (95% CI 1.3 to 2.0) for each 100-pmol/L increase in C-peptide.

Because insulin and C-peptide were highly correlated (Spearman $r = 0.84$), we examined the prevalence of IR by tertiles of insulin and C-peptide (Fig. 1). In each insulin tertile, the fraction of participants with IR increased according to C-peptide tertile level. Similarly, at each C-peptide tertile, the fraction of participants with IR increased according to insulin tertile level. For example, in the medium tertile of insulin, IR was present in 10% of subjects whose C-peptide concentration was in the low tertile compared with in ~43% of those whose C-peptide concentration was in the high tertile. Collectively, these observations demonstrate that insulin and C-peptide concentrations were both independently associated with IR.

We used a stepwise selection procedure to identify variables for inclusion in an IR score. When we used $P < 0.001$ as the significance level for both variable inclusion and retention in the score, insulin, C-peptide, and creatinine were included (in that order). For those in the top quartile of a score comprising insulin, C-peptide, and creatinine (vs those not being in the top quartile), the odds ratio was 10.9 (95% CI 6.2 to 19.0) after adjustment for all variables that were not included in the score. When the significance level for variable inclusion and retention was relaxed ($P < 0.05$), five variables were included: insulin, C-peptide, creatinine, BMI, and TG/HDL-C (in that order). For individuals in the top quartile of this five-variable score, the adjusted odds ratio for IR was 16.1 (95% CI 9.5 to 27.3). Because insulin and C-peptide can be measured in a single multiplexed test, we also examined a score that included only insulin and C-peptide and found that the adjusted odds ratio for IR was 6.9 (95% CI 3.9 to 12.1) for those in the top quartile of the score compared with those who were not. All three scores were associated with IR in both those with and without metabolic syndrome (Supplemental Table 2). When these scores and HOMA-IR were compared on an odds ratio per SD basis, the odds ratio of IR ranged from 2.4 (95% CI 1.4 to 4.0) for HOMA-IR to 11.4 (95% CI 7.0 to 18.5) for the five-variable score (Table 2).

These scores can be converted to the probability of being insulin resistant (see equations in the Supplemental Appendix). For those with a probability score >66% (compared with those with <33% probability score), the odds ratio was 15.6 (95% CI 7.5 to 32.4; Table 3) for a score comprising insulin and C-peptide and 26.7 (95% CI 14.0 to 50.8) for a score comprising insulin, C-peptide, creatinine, TG/HDL-C, and BMI.

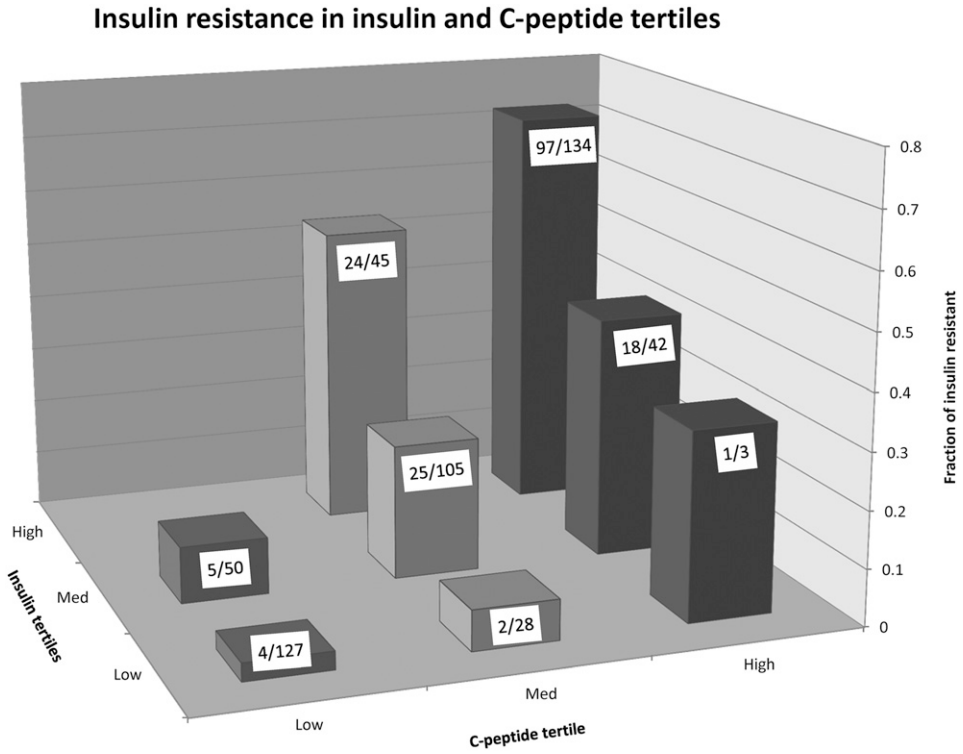

Figure 1. IR according to insulin and C-peptide tertile. The fraction of individuals having IR at each of the nine tertile combinations of insulin and C-peptide is indicated on each bar (number of those with IR/total number).

3. Discussion

The aim of this study was to develop a clinically practical probability score to assess IR in apparently healthy individuals. We directly assessed IR by measuring steady-state glucose levels during the insulin-suppression test. We generated probability scores for IR based on insulin and C-peptide levels determined by a mass-based assay as well as creatinine, TG/HDL-C, and BMI.

A number of variables were associated with IR when they were adjusted for only for age, sex, and ethnicity. These variables included FG, insulin, C-peptide, TG/HDL-C ratio, ALT, BMI, SBP, and DBP. In addition, those with self-reported Hispanic ethnicity had 2.5 greater

Table 2. Association Between IR and Biomarkers and Scores

	Per SD		Top Quartile vs Not	
	OR (95% CI)	***P* Value**	**OR (95% CI)**	***P* Value**
HOMA-IR	2.4 (1.4–4.0)	0.001	1.5 (0.8–2.7)	0.2
Insulin	2.5 (1.5–4.2)	6×10^{-4}	1.6 (0.8–3.0)	0.15
C-peptide	3.2 (1.9–5.3)	1×10^{-5}	2.2 (1.1–4.3)	0.02
Insulin and C-peptide	7.2 (4.6–11.5)	4×10^{-17}	6.9 (3.9–12.1)	2×10^{-11}
Insulin, C-peptide, and creatinine	7.2 (4.6–11.2)	1×10^{-17}	10.9 (6.2–19.0)	6×10^{-17}
Insulin, C-peptide, creatinine, TG/HDL-C, and BMI	11.4 (7.0–18.5)	7×10^{-23}	16.1 (9.5–27.3)	1×10^{-26}

Score coefficients were estimated in models that adjusted for all study variables not included in the score. Insulin and C-peptide were estimated in models that include age, sex, ethnicity, insulin, C-peptide, creatinine, BMI, TG/HDL-C, FG, SBP, DBP, LDL-C, and ALT. Top quartile of insulin: >71.64 pmol/L. Top quartile of C-peptide: >652.611 pmol/L. Top quartile of HOMA-IR: >2.9926.
Abbreviation: OR, odds ratio.

Table 3. Association Between IR and Predicted Probability of IR

	IR Probability			
	33% to 66%		>66%	
Score	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Insulin and C-peptide	4.4 (2.5–7.8)	5×10^{-7}	15.6 (7.5–32.4)	2×10^{-13}
Insulin, C-peptide, and creatinine	4.5 (2.5–7.9)	2×10^{-7}	17.7 (9.0–34.8)	6×10^{-17}
Insulin, C-peptide, creatinine, TG/HDL-C, and BMI	7.3 (4.2–12.5)	7×10^{-13}	26.7 (14.0–50.8)	2×10^{-23}

Odds ratios (OR) are vs those with <33% probability. The probability of IR for each participant was calculated using the equations in the Supplemental Appendix. The OR for those with >66% probability (or those with 33% to 66% probability) vs those with <33% probability was estimated in models that adjusted for all study variables not included in the score.

odds of IR than non-Hispanic whites. However, only insulin, C-peptide, TG/HDL-C ratio, BMI, and creatinine were associated with IR in a fully adjusted model.

An unexpected finding of this study was that insulin and C-peptide were both independently associated with IR (Fig. 1). Fasting insulin levels are expected to be correlated with IR because, to compensate for IR, one of the initial physiological responses is an increase in steady-state fasting insulin level resulting from an increased insulin secretion rate. However, the independent association of insulin levels and C-peptide levels with IR was unexpected because insulin and C-peptide are derived from the same precursor peptide and are cosecreted by pancreatic β cells in an equimolar ratio. Although we cannot be certain of the underlying mechanism that explains the independent association of insulin and C-peptide with IR, the different clearance pathways of insulin and C-peptide could explain this independent association. Insulin clearance rates can change substantially as IR develops and are also influenced by BMI and possibly liver steatosis [18]. C-peptide clearance, which occurs primarily in the kidney, will depend on kidney function [19, 20]. Given these different clearance pathways and the modifiable rate of insulin clearance, it may be that the insulin and C-peptide levels, either alone or in combination, become unexpectedly disproportionate compared with the degree of IR in some individuals; therefore, the combination of insulin and C-peptide levels provides more information about IR than either does alone. This would also be consistent with both BMI and creatinine entering the model during stepwise model building. Plasma creatinine, a marker for glomerular filtration rate, may provide information about C-peptide clearance rates in the kidney, and BMI may provide information about differences in the insulin clearance rate.

Because IR has been demonstrated to be an early predictor of future diabetes and other clinical syndromes [2, 3, 5], we believe that clinicians would find it useful to assess IR for three reasons: first, it would help assess risk of diabetes in patients who are not prediabetic, but who have other reasons to be concerned about their risk for future diabetes, such as statin therapy or East Asian or South Asian ancestry. Second, identifying patients with a high probability of IR would help identify those who would most benefit from preventative efforts and motivate both patients and clinicians to consider intensive lifestyle programs such as weight loss and exercise to mitigate IR. Finally, it would allow clinicians to monitor the success of their efforts to prevent diabetes and diseases associated with IR.

This study has several limitations. First, we defined IR as the top tertile of the SSPG distribution in this study. This definition is based on previously observed risk of future disease among those in the top tertile of SSPG [4, 7]. However, some have suggested that the top quartile of plasma insulin response to a glucose challenge is also a reasonable way to define IR [5]. We chose the top SSPG tertile to define IR because, if left uncorrected, IR can lead to serious morbidity [4, 7]. Second, we did not consider additional variables for inclusion in an IR probability score. In particular, hemoglobin A1C, which has been shown to be a predictor of diabetes independent of FG [21, 22], could be assessed as a component of IR

probability score in future studies. Third, although this study did include participants from multiple ethnic groups, the number of participants from some of these groups was limited. Additional studies using different populations would help extend the findings reported in this study. Finally, we acknowledge that direct measurement of IR, when feasible, would likely improve patient assessment. In contrast, a major strength of this study is that we quantified IR directly by measuring SSPG levels during an insulin-suppression test in a large study of apparently healthy individuals.

4. Conclusion

We have demonstrated that a score based on fasting insulin, C-peptide, creatinine, BMI, and TG/HDL-C can be used to assess the probability of being insulin resistant. The simplicity of the score and the use of mass-based, standard traceable measurement of insulin and C-peptide can be used to establish a standard assessment of IR across populations. Our findings suggest that IR probability scores may be valuable for the longitudinal assessment of subjects engaged in lifestyle or pharmacological interventions to decrease IR.

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Author Contributions: F.A. was responsible for study design and reviewed and edited the manuscript. D.S. was responsible for study design, drafted the manuscript, and takes full responsibility for the work as a whole. C.H.T. analyzed the data and reviewed and edited the manuscript. J.J.D. was responsible for study design and reviewed and edited the manuscript. M.J.M. was responsible for study design and reviewed and edited the manuscript.

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Disclosure Summary: D.S., C.H.T., J.J.D., and M.J.M. are employees of Quest Diagnostics. The remaining author has nothing to disclose.

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