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Predictive Accuracy of Surrogate Indices for Hepatic and Skeletal Muscle Insulin Sensitivity

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Context: Surrogate indices of muscle and hepatic insulin sensitivity derived from an oral glucose tolerance test (OGTT) are frequently used in clinical studies. However, the predictive accuracy of these indices has not been validated.

Design: In this cross-sectional study, hyperinsulinemic-euglycemic glucose clamp with tritiated glucose infusion and a 75-g OGTT were performed in individuals (n = 659, aged 18 to 49 years, body mass index of 16 to 64 kg/m^2) with varying degrees of glucose tolerance. A calibration model was used to assess the ability of OGTT-derived, tissue-specific surrogate indices [hepatic insulin resistance index (HIRI) and muscle insulin sensitivity index (MISI)] to predict insulin sensitivity/resistance indices derived from the reference glucose clamp [Hepatic-IR$_{basal}$, a product of fasting plasma insulin and hepatic glucose production (HGP), Hepatic-IR$_{clamp}$, reciprocal of the percent suppression of HGP during the insulin clamp corrected for plasma insulin concentration, and Muscle-IS$_{clamp}$, a measure of peripheral glucose disposal]. Predictive accuracy was assessed by root mean squared error of prediction and leave-one-out, cross-validation-type square root of the mean squared error of prediction.

Results: HIRI and MISI were correlated with their respective clamp-derived indices. HIRI was negatively related to Muscle-IS$_{clamp}$ ($r = –0.62$, $P < 0.0001$) and MISI correlated with Hepatic-IR derived from the clamp (Hepatic-IR$_{basal}$: $r = –0.48$, $P < 0.0001$ and Hepatic-IR$_{clamp}$: $r = –0.41$, $P < 0.0001$). However, the accuracy of HIRI and MISI to predict Hepatic-IR (basal or during clamp) was not significantly different. Likewise, the ability of HIRI and MISI to predict Muscle-IS$_{clamp}$ was also similar.

Conclusion: Our findings indicate that the surrogate indices derived from an OGTT are accurate in predicting insulin sensitivity but are not tissue specific.

Insulin resistance is typically defined as decreased sensitivity to metabolic actions of insulin, including peripheral glucose disposal and suppression of hepatic glucose production (HGP) [1–3]. Skeletal muscle and hepatic insulin resistance play a major pathophysiological role in type 2 diabetes and is frequently observed in obesity, hypertension, dyslipidemia, coronary heart disease, and the metabolic syndrome [4, 5]. In insulin-resistant individuals, insulin action is impaired at multiple sites, including the liver, muscle, adipose tissue, and the

Abbreviations: AUC, area under the curve; CVPE, leave-one-out cross-validation-type root mean squared error of prediction; HGP, hepatic glucose production; HIRI, hepatic insulin resistance index; IGT, impaired glucose tolerance; MISI, muscle insulin sensitivity index; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; RMSE, square root of the mean squared error of prediction.

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vasculature [3, 6]. Skeletal muscle insulin resistance is characterized by reduced insulin-mediated glucose disposal and contributes to postprandial hyperglycemia [7]. Impaired insulin-mediated suppression of HGP, a feature of hepatic insulin resistance, is partly due to enhanced gluconeogenesis and reduced inhibition of glycogenolysis that leads to fasting hyperglycemia [8]. In fact, hepatic and muscle insulin resistance differentially affect the development of impaired fasting glucose and postprandial glucose intolerance [9, 10].

Lifestyle interventions and weight reduction improve insulin action, thereby decreasing the progression to type 2 diabetes [11, 12]. Some pharmacological interventions (*e.g.*, metformin and thiazolidinediones) also improve insulin action and reduce the risk of conversion from impaired glucose tolerance (IGT) to type 2 diabetes [11]. However, these interventions appear to modulate hepatic and skeletal muscle insulin sensitivity differently [13–15]. Therefore, accurate tissue-specific metabolic phenotyping and quantitation of the presence and severity of insulin resistance, particularly in nondiabetic/prediabetic subjects, to identify high-risk individuals and initiate intervention programs are important.

The "hyperinsulinemic euglycemic glucose clamp" is widely accepted as the reference method to evaluate insulin sensitivity because it can directly measure insulin-mediated suppression of HGP and whole-body glucose disposal [16]. However, the glucose clamp is labor intensive, technically demanding, and time consuming, thus precluding its use in epidemiological studies. Consequently, surrogate indices are extensively used to quantify insulin action [2]. After an oral glucose challenge, the HGP is maximally suppressed reaching a nadir at approximately 60 minutes and remains suppressed for the duration (~180 minutes) [17, 18]. Therefore, glucose uptake by peripheral tissues (*e.g.*, muscle and adipose tissue) primarily determines the rate of decrease in plasma glucose concentration from its peak value to its nadir during an oral glucose tolerance test (OGTT). Based on this observation, Abdul-Ghani *et al.* [19] developed surrogate indices of hepatic and muscle insulin sensitivity/resistance from an OGTT that has been widely used. However, the predictive accuracy of these indices has not been examined. In this study, we compared the ability of these surrogate indices to accurately predict tissue-specific insulin sensitivity as determined by the reference glucose clamp method.

1. Research Design and Study Methods

A total of 659 volunteers participating in a longitudinal study of predictors of type 2 diabetes were included in this study [20, 21]. This subset included individuals in their first visit who had complete data on OGTT, insulin action measured by the gold-standard glucose clamp technique, and body composition measured by either underwater weighing with simultaneous determination of residual lung volume by helium dilution [22] or dual energy x-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) [23]. The absorptiometry measures were converged to comparable underwater weighing values, using previously derived equations to calculate body fat percentage [24]. All subjects provided written informed consent for their participation. The study was approved by the Institutional Review Board, National Institute of Diabetes & Digestive & Kidney Diseases. Ethnicity of the participants was identified by self-report. All subjects underwent laboratory testing, history, and physical examination to rule out any other medical disorders. Subjects were not on any medications and were nonsmokers. No subjects had a history of chronic viral hepatitis or liver disease. Subjects were abstinent from alcohol use for at least 2 weeks. Upon admission, subjects were fed a weight-maintaining diet (caloric distribution: 50% carbohydrates, 30% fat, and 20% protein), abstained from strenuous activities, and underwent an OGTT and glucose clamp after at least 3 days of weight-maintaining diet.

A. OGTT

After an overnight fast, a 75-g oral glucose load was given. Blood samples drawn at time 0-, 30-, 60-, 120-, and 180-minute time intervals for measurement of plasma glucose and insulin concentrations. Depending on the results of the OGTT, subjects were categorized as either having normal glucose tolerance (NGT), IGT, or type 2 diabetes mellitus per American

Diabetes Association 2003 criteria [25]. Plasma insulin concentrations were measured by three different radioimmunoassays over time: the modified Herbert-Lau assay, Concept 4 (ICN, Costa Mesa, CA), and Access (Beckman Instruments, Fullerton, CA). Through regression equations, all measurements of plasma insulin were normalized to the original radioimmunoassay (modified Herbert-Lau assay). Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments).

B. HIRI and MISI

OGTT-derived, tissue-specific surrogate indices for insulin sensitivity were derived as previously reported [19]. The hepatic insulin resistance index (HIRI) was calculated as the product of the glucose area under the curve (AUC; $mg/min^{-1}/dL$) and insulin AUC ($\mu U/min^{-1}/mL$) during the first 30 minutes during the OGTT. The muscle insulin sensitivity index (MISI) was derived by dividing the rate of decline in plasma glucose concentration, calculated as the slope of the decrease in plasma glucose concentration (dG/dt) from peak to nadir, by the mean plasma insulin concentration and expressed as 10^{-2} $[(mg/dL/min^{-1})/(\mu U/mL)]$.

C. Hyperinsulinemic-Euglycemic Glucose Clamp

Insulin action was evaluated by glucose clamp [21]. After an overnight fast of ≥10 hours, clamp studies were performed the following morning. In postabsorptive states, ambient plasma insulin suppresses hepatic endogenous glucose production (HGP). Impairment in this action indicates hepatic insulin resistance. Thus, the product of basal HGP and plasma insulin concentration is a measure of hepatic insulin resistance. HGP was determined by intravenous 3-[^{3}H] glucose infusion [primed bolus (1.11 MBq) followed by continuous infusion (0.0111 MBq/min) for 2 hours]. Basal HGP was calculated during the fasting state as the 3-[^{3}H] glucose infusion rate divided by the steady-state plasma 3-[^{3}H] glucose-specific activity (measured with Beckman LS6500 scintillation counter; Beckman Instruments). Fasting plasma insulin levels were measured as previously described. Hepatic-IR_{Basal} is thus the product of HGP and fasting plasma insulin [26]. Although performed at the beginning of a clamp procedure, this direct measure of hepatic sensitivity is not strictly clamp based and is not directly dependent on the insulin infusion. Two hours after beginning the infusion of 3-[^{3}H] glucose, a 10-minute priming dose of insulin was administered followed by a continuous infusion at a constant rate ($40\ mU/m^2/min^{-1}$). Plasma glucose concentrations from arterialized blood samples were measured at the bedside every 5 minutes with a glucose analyzer. An intravenous infusion of dextrose was adjusted to maintain the plasma glucose concentration ~100 mg/dL. Mean parameter values were used to calculate SI_{clamp} [defined as M/I corrected for estimated metabolic body size (fat free mass + 17.7), where M is the glucose infusion rate (mg/min) and I is the steady-state plasma insulin concentrations (μU/mL)] [27]. The rate of glucose disposal (M) was defined as the average of the glucose infusion rate during the last 40 minutes of the insulin infusion. The measure SI_{clamp} represents insulin-mediated peripheral glucose disposal and signifies predominantly skeletal muscle insulin sensitivity (Muscle-IS_{clamp}). During the insulin clamp, steady-state HGP was the difference between the appearance rates of glucose in the plasma calculated from 3-[^{3}H] glucose measurements during insulin infusion and the glucose infusion rate. Hepatic-IR_{clamp}, an additional index of hepatic insulin resistance during the clamp, was derived as the reciprocal of the percent suppression of HGP during the insulin clamp corrected for plasma insulin concentration **(**$[1/(100 \times (HGP_{basal}–HGP_{clamp})/HGP_{basal} \div I]$**)**.

D. Statistical Analysis

All indices were log transformed to approximate normality. Subject characteristics are depicted as mean ± SD or median (25th to 75th percentile). Normally distributed variables were analyzed by Student *t* test and for multiple groups by one-way ANOVA. Linear regression models were used to calculate least square means and 95% CI for various insulin sensitivity/resistance indices after adjusting for age and sex. We used a calibration model to assess the ability of these tissue-specific surrogate indices derived from an OGTT to predict

insulin sensitivity measures derived from a clamp [28, 29]. Using calibration models is particularly appropriate when an accurate measurement method, such as the glucose clamp, is compared with an indirect method such as MISI and HIRI. Calibration is inverse regression in which the surrogate method is regressed on the accurate measurement method and new x* (clamp derived) is predicted for a given y* (surrogate derived). A calibration model, $(SI_{clamp})_i = \alpha + \beta(OGTT\text{-}surrogate)_i + \varepsilon_i$, where ε_i is random error for the ith subject, was fitted. Random error having a Gaussian distribution with $\mu = 0$ and constant variance was assumed. Two types of predicted residuals were considered: The first type of residual was the difference between measured clamp variable (x_i for the ith subject) and fitted SI_{clamp} ($\hat{x_i} = \alpha + \beta y_i$) with all subjects included in the estimation of model parameters α and β. The second type of residual considered is a cross-validation-type predicted residual $e_{(i)} = x_i - \hat{x_{(i)}}$, where x_i is still the measured clamp but $\hat{x_{(i)}}$ is the predicted clamp from the calibration model that excludes the ith subject. Two measures of predictive accuracy were calculated from these residuals: square root of the mean squared error of prediction (RMSE) and leave-one-out cross-validation-type root mean squared error of prediction (CVPE). Smaller values indicate better predictive power, and CVPE is more robust than RMSE because CVPE uses an estimate that excludes the ith subject when predicting results for the ith subject. To compare predictive accuracy of the surrogates in terms of CVPE/RMSE, the one-sided alternative hypotheses that HIRI had a smaller RMSE/CVPE than MISI for hepatic insulin resistance and *vice versa* for muscle insulin resistance were established. We used a bootstrap percentile method with 60,000 replications performed for each comparison. The RMSEs (or CVPEs) corresponding to surrogate indices were derived from the same group of subjects and thus the bootstrap method is appropriate. The P values calculated from comparisons of RMSE and CVPE were for pairwise comparisons.

2. Results

Baseline demographics, clinical characteristics, glucose metabolism indices, and measurements of insulin sensitivity of our study subjects are summarized in Table 1. In models adjusted for age and sex, basal HGP was higher in patients with type 2 diabetes mellitus, but hepatic insulin resistance as determined by Hepatic-IR_{basal}, Hepatic-IR_{clamp}, and HIRI was

Table 1. Characteristics of Study Participants

	NGT (n = 446)	IGT (n = 188)	T2DM (n = 25)	*P* Value
Age, y	27 ± 6	29 ± 7	30 ± 7	<0.05
Female (%, n)	36% (n = 159)	54% (n = 102)	64% (n = 16)	<0.0001
Body mass index, kg/m^2	32 ± 8^a	37 ± 8^b	39 ± 9^c	<0.0001
Body fat (%)	30 ± 9^a	36 ± 8^b	38 ± 8^b	<0.0001
Metabolic parameters				
Fasting plasma glucose, mg/dL	86 ± 7^a	97 ± 10^b	128 ± 36^c	<0.0001
Fasting plasma insulin, pmol/L	30 (22)^a	47 (30)^b	57 (41)^b	<0.0001
2-h plasma glucose, mg/dL	105 ± 20^a	150 ± 23^b	239 ± 54^c	<0.0001
Indices of insulin sensitivity				
Basal HGP (mg/kg$_{EMBS}$/min)	1.9 (0.3)^a	1.9 (0.3)^a	2.1 (0.8)^b	<0.0001
Hepatic-IR_{basal} (mg/kg^{-1}/min^{-1}/μU/mL)	55 (40)^a	82 (56)^b	112 (74)^c	<0.0001
Hepatic-IR_{clamp}	1.47 (0.93)^a	1.85 (1.03)^b	2.29 (1.79)^b	<0.0001
HIRI	11.40 (10.26)^a	15.66 (10.54)^b	15.18 (13.23)^b	<0.0001
Muscle-IS_{clamp} 10^{-3} (mg/kg^{-1}/min^{-1})/(μU/mL)	21.13 (18.43)^a	15.74 (9.87)^b	12.87 (9.87)^b	<0.0001
MISI	14.93 (15.30)^a	12.87 (11.27)^b	15.57 (16.31)^a	<0.0001

Data are presented as mean ± SD or median (interquartile range). HIRI is measured as 10^6 [glucose AUC (mg/min^{-1}/dL) × insulin AUC (μU/min^{-1}/mL) during the first 30 min of the OGTT], and MISI is measured as 10^{-2} (mg/dL/min^{-1})/(μU/mL). One-way ANOVA followed by Tukey *post hoc* test was used to compare differences between groups. *P* values are adjusted for age and sex. Means or medians sharing the same superscript letter are not significantly different from each other across glucose tolerance status ($P < 0.05$)

Abbreviations: EMBS, estimated metabolic body size (fat free mass + 17.7); T2DM, type 2 diabetes mellitus.

significantly higher in subjects with IGT and type 2 diabetes mellitus. Similarly, muscle insulin sensitivity as determined by the glucose clamp was significantly lower in IGT and type 2 diabetes mellitus when compared with NGT individuals. MISI was lower in the IGT group but not significantly different than NGT. In terms of racial composition, our study population was comprised of American Indians (n = 516), African Americans (n = 33), and white individuals (n = 110). Hepatic-IR_{basal}, Hepatic-IR_{clamp}, and HIRI were significantly higher, and Muscle-IS_{clamp} and MISI were significantly lower, in American Indians when compared with African Americans and white individuals (data not shown).

When we compared relationships between tissue-specific surrogate indices and clamp-derived indices, simple linear regression analysis showed modest correlations between Hepatic-IR and HIRI (Hepatic-IR_{basal} vs HIRI: $r = 0.57$, $P < 0.0001$ and Hepatic-IR_{clamp} vs HIRI: $r = 0.49$, $P < 0.001$) and between Muscle-IS_{clamp} and MISI ($r = 0.50$, $P < 0.0001$). Indices of hepatic insulin resistance were negatively associated with indices of muscle insulin sensitivity (Muscle-IS_{clamp} vs Hepatic-IR_{basal}: $r = -0.78$, $P < 0.0001$; Muscle-IS_{clamp} vs Hepatic-IR_{clamp}: $r = -0.83$, $P < 0.0001$; Muscle-IS_{clamp} vs HIRI: $r = -0.62$, $P < 0.0001$; MISI vs Hepatic-IR_{basal}: $r = -0.48$, $P < 0.0001$; MISI vs Hepatic-IR_{clamp}: $r = -0.41$, $P < 0.0001$; and MISI vs HIRI: $r = -0.53$, $P < 0.0001$). Sex did not modulate the strength of these relationships. A significant portion of the study cohort (~78%) are American Indians who are well known to be insulin resistant [30]. The primary purpose of these surrogate indices of insulin sensitivity is to recognize and characterize tissue-specific insulin resistance. Therefore, we examined if the insulin resistance status affected the relationship between clamp-derived measure and surrogate index. Based on prior clamp studies that use an insulin infusion rate of 40 $mU/m^2/min^{-1}$, we defined insulin resistance as an M value expressed as a function of metabolic size (FFM + 17.7) < 3.6 mg/FFM + 17.7*min [31, 32]. Based on this criterion, 170 and 489 subjects were characterized as insulin sensitive and insulin resistant, respectively. We then examined if the insulin resistance status modified the linear relationships between Muscle-IS_{Clamp} and MISI. The correlation coefficients between Muscle-IS_{clamp} and MISI were similar in the insulin-resistant ($r = 0.39$, $P < 0.0001$) and insulin-sensitive groups ($r = 0.35$, $P < 0.0001$).

The absolute accuracy of the tissue-specific surrogate indices derived from OGTT were assessed using the calibration model. Experimentally determined hepatic IR and muscle IS from the clamp studies were regressed on each surrogate index, and data were fit with a calibration model. Using the leave-one-out cross-validation analysis, we used the fitted calibration model to generate plots for each surrogate index (HIRI and MISI), comparing predicted clamp values (generated from each surrogate index) with actual values for each subject (Fig. 1). Perfect prediction by a surrogate index would result in predicted values along a straight line with a slope of 1 and a y-intercept of 0. Both HIRI and MISI predict their respective clamp-derived indices reasonably well. However, as seen in Fig. 1(B), 1(D), and 1(E), MISI and HIRI predicted Hepatic-IR_{clamp} and Muscle-IS_{clamp}, respectively, as well. These results suggest that although the surrogate indices accurately predict insulin sensitivity/resistance, they are not tissue specific.

Further, we have calculated cross-validation-type error (CVPE) and root mean square analysis (RMSE) from calibration analysis to quantitatively assess prediction errors of these surrogate indices of insulin sensitivity/resistance. RMSE and CVPE analysis summarized in Table 2 reveals that both MISI and HIRI equally predict the insulin resistance at the liver and muscle tissues, with the same precision. These results together suggest that although the surrogate indices accurately predict insulin sensitivity/resistance, they are not tissue specific.

3. Discussion

In this study, we examined the absolute predictive accuracy of tissue-specific insulin sensitivity/resistance indices derived from an OGTT by comparing it with corresponding glucose clamp estimates. HIRI and MISI are reasonably accurate in predicting Hepatic-IR_{clamp} and Muscle-IS_{clamp}, respectively. However, MISI was as accurate as HIRI in

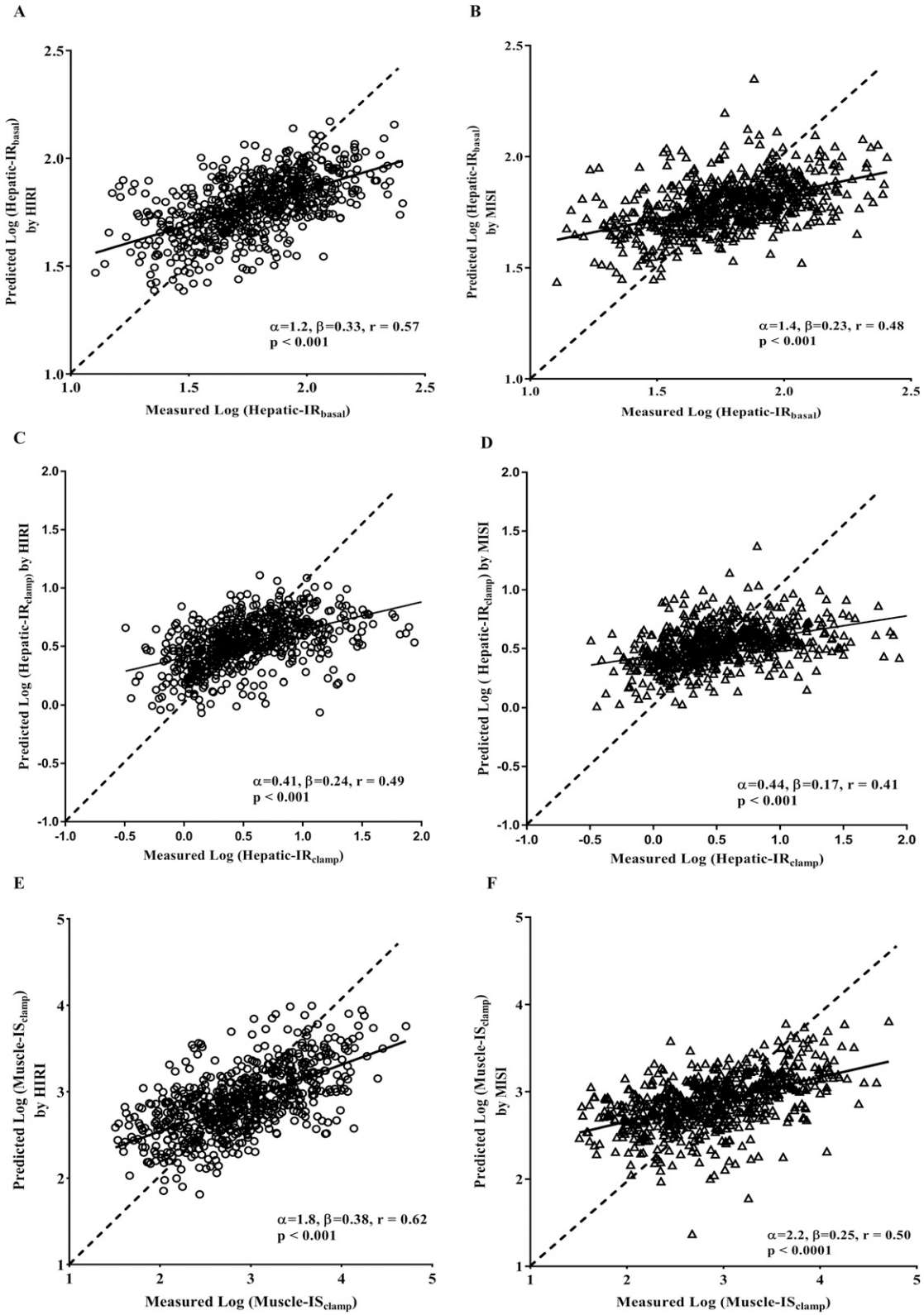

Figure 1. Comparison between measured and predicted clamp insulin sensitivity/resistance measures (Hepatic-IR$_{basal}$, Hepatic-IR$_{clamp}$, and Muscle-IS$_{Clamp}$) from tissue-specific surrogate indexes of insulin sensitivity/resistance. Predicted liver or muscle reference measures shown for each surrogate index were calculated using the leave-one-out cross-validation analysis of the calibration model as described in the research design and methods. The dashed line

indicates ideal predictive accuracy. The solid line indicates the linear least-squares fit of measured vs predicted liver or muscle measure. Correlation coefficients (r) and respective P values are shown in each panel. (A, C, and E) Results derived from HIRI. (B, D, and F) Results derived from MISI.

predicting Hepatic-IR_{clamp}. Likewise, HIRI and MISI predicted Muscle-IS_{clamp} with similar accuracy. These findings suggest that surrogate indices derived from an OGTT, HIRI, and MISI may not be tissue-specific as originally proposed [19].

Insulin resistance, a characteristic feature of type 2 diabetes [33] [34], is associated with obesity and atherosclerotic cardiovascular disease [4]. As impaired insulin action is the cornerstone for the development of type 2 diabetes and metabolic syndrome, there has been widespread interest in the development of techniques and methods to assess insulin sensitivity [2]. Simple, feasible, reliable, and accurate methods for quantifying insulin sensitivity is necessary to identify insulin-resistant individuals [2]. Hepatic and muscle insulin sensitivity can be simultaneously measured during a glucose clamp when used in combination with radiolabeled glucose [35]. Because of the nature of the clamp study, it is not a feasible option for large studies. Therefore, to quantitate insulin sensitivity, several simpler methods were derived from the OGTT [35], a commonly used test to assess glucose homeostasis in clinical practice, epidemiological studies, and research settings. Based on the kinetics of HGP and peripheral glucose disposal during an OGTT, Abdul-Ghani *et al.* [19] developed indices of hepatic and skeletal muscle insulin sensitivity in nondiabetic subjects. The study has been cited over 200 times and these indices have been used in multiple studies and conclusions are based on the assumed tissue "selectivity" of these indices [36–40].

Our study cohort was large (n = 659) with normal healthy subjects and subjects with varying degrees of obesity and glucose intolerance. In addition, there was a wide range of hepatic insulin resistance and muscle insulin sensitivity. The utility of the OGTT-derived surrogate indices is primarily in insulin-resistant subjects, especially following interventions or in large cross-sectional studies. Therefore, examining the accuracy of these indices in this cohort with a substantial portion of insulin-resistant subjects does not affect robustness of the calibration analysis.

The study by Abdul-Ghani *et al.* [19] included Mexican-American subjects (n = 155) who received a euglycemic-hyperinsulinemic clamp (40 $mU/m^2/min^{-1}$) and a 75-g OGTT. In that study, OGTT-derived MISI strongly correlated with insulin sensitivity, measured with the hyperinsulinemic-euglycemic clamp ($r = 0.78$, $P < 0.0001$). Similarly, HIRI was significantly related to a direct measure of hepatic insulin resistance ($r = 0.64$, $P < 0.001$). In a sample of nondiabetic individuals (n = 368) from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study, Vangipurapu *et al.* [41] have also examined the relationship between HIRI and Hepatic-IR_{basal}. They report that HIRI was directly related to Hepatic-IR_{basal} with a correlation coefficient of $r = 0.58$. The correlation coefficient ($r = 0.57$) between Hepatic-IR_{basal} and HIRI in our study is similar in magnitude to reported correlation

Table 2. CVPE and RMSE Calculated From Calibration Analysis of Tissue-Specific Surrogate Indices of Insulin Sensitivity/Resistance

	RMSE Hepatic-IR_{basal}	CVPE Hepatic-IR_{basal}	RMSE Hepatic-IR_{clamp}	CVPE Hepatic-IR_{clamp}	RMSE Muscle-IS_{clamp}	CVPE Muscle-IS_{clamp}
HIRI	0.20	0.20	0.37	0.37	0.48	0.48
MISI	0.21	0.21	0.39	0.39	0.54	0.54
P value	0.96	1.00	0.95	1.00	0.99	0.48

CVPE and RMSE were calculated from calibration analysis of tissue-specific surrogate indices of insulin sensitivity derived from an OGTT as described in the research design and methods. P values correspond to comparisons between the surrogate indexes.

coefficients in studies by Abdul-Ghani *et al.* [19] and Vangipurapu *et al.* [41]. The study by Vangipurapu *et al.* did not examine the relationship between MISI and insulin sensitivity as measured with the hyperinsulinemic-euglycemic clamp technique. However, Bastard *et al.* [42] report a modest but significant association between MISI and a glucose clamp-derived insulin sensitivity measure ($r = 0.54$) in older, nondiabetic postmenopausal women (n = 113). The magnitude of the relationship between Muscle-IS_{clamp} and MISI observed in our study ($r = 0.49$, $P < 0.0001$) is not significantly different than Bastard *et al.* [42]. In the study by Ghani *et al.* [19], HIRI correlated not only with Hepatic-IR_{basal} ($r = 0.64$), but also with Muscle-IS_{clamp} ($r = –0.55$). Similarly, MISI was related to Muscle-IS_{clamp} ($r = 0.78$) and Hepatic-IR_{basal} ($r = 0.46$). Based on the differences in correlation coefficients, $r = 0.78$ vs $r = 0.46$ for MISI and $r = 0.64$ vs $r = 0.55$ for HIRI, the authors concluded that the proposed indices had "greater selectivity" in detecting muscle and hepatic insulin sensitivity, respectively [19].

Magnitude of correlation coefficients are not particularly informative about predictive ability, because it is possible to observe a very strong correlation with almost zero predictive accuracy. Furthermore, if the sample is not normally distributed, the observed correlation overestimates the predictive accuracy. Measures of insulin sensitivity/resistance are typically not normally distributed, and the results of simple correlational analyses in such a sample do not inform predictive ability. Thus, assessing predictive accuracy is an important aspect of validating a surrogate index. The relationships between surrogate indices and the clamp-derived measures were modest in our current study as well as by the study of Abdul-Ghani *et al.* [19]. However, using calibration model analysis, we found that CVPE and RMSE, criterion functions measuring predictive accuracy, were not different among the surrogate indices (HIRI and MISI). These findings clearly suggest that these surrogate indices are not tissue specific. Furthermore, insulin resistance in liver and muscle are strongly associated [1]. Therefore, the cross-correlation between hepatic and muscle indices may reflect the underlying physiology rather than limitations of the indices themselves. Nevertheless, the use of HIRI and MISI to evaluate changes in insulin sensitivity "specifically" in the liver or skeletal muscle is not supported by our findings.

There are many strengths to our study. The analyses are from a large data set, which includes American Indians, African Americans, and white individuals and thus is more diverse. Both American Indians and African Americans are at high risk for developing type 2 diabetes mellitus. The study cohort also includes individuals with a wide range of body fat content, glucose tolerance, and insulin sensitivity. Insulin sensitivity was measured using the reference clamp technique along with the use of a isotope dilution technique to measure HGP. When comparing Muscle-IS_{clamp} indices from different glucose clamp studies, it is imperative that the insulin doses used in the clamps are comparable. The original study by Abdul-Ghani *et al.* [19] in which these surrogate indices were first proposed used an insulin dose of 40 $mU/m^2/min^{-1}$, similar to the dosage in our study. HIRI is calculated in an insulin-stimulated state (*i.e.*, during an OGTT), whereas Hepatic-IR_{basal} is measured during fasting. Therefore, we also examined the relationship between the clamp-derived measure of hepatic insulin sensitivity and HIRI. Lastly, we used a robust calibration model to validate these indices and assess predictive accuracy. Among the weaknesses, our cohort is mainly comprised of Pima Indians who are highly insulin resistant. Therefore, one could argue that lower glucose disposal rates especially with a clamp insulin dose of 40 $mU/m^2/min^{-1}$ may thus have contributed to the less-than-robust relationship between the surrogate index and Muscle-IS_{clamp}. However, the strength of the relationship between MISI and Muscle-IS_{clamp} did not differ in subjects classified as "insulin sensitive" or "insulin resistant," suggesting that there was no procedural bias. Furthermore, our cohort was more heterogeneous, whereas the population was comprised of Mexican Americans in the original study by Abdul-Ghani *et al.* [19] and of postmenopausal white women in the Canadian study [42]. OGTT-derived indices generally have high within-subject variability [43]. Calculation of MISI involves multiple time points for glucose and insulin and thus is more prone for more measurement error. This is clearly evident in the rank order of tissue-specific insulin resistance using the clamp or surrogate index in the subjects with NGT, IGT, and type 2 diabetes mellitus (Table 1). MISI

was clearly lower in the IGT group, but was unable to distinguish NGT and type 2 diabetes mellitus groups. Perhaps the small sample size of type 2 diabetes mellitus in our cohort contributed to this finding. Finally, because insulin assays are not standardized, there are no cutoff points for these tissue-specific surrogate indices of insulin resistance.

In conclusion, our findings indicate that HIRI and MISI surrogate indices are not tissue specific as previously proposed. Our study findings caution the use and interpretation of OGTT-derived surrogate markers specifically to evaluate hepatic or muscle insulin sensitivity in large epidemiological studies. Finally, further research on surrogate indices that are feasible and accurately predict tissue-specific insulin sensitivity/resistance are needed for use in clinical studies.

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