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Surrogate measures of insulin sensitivity when compared to euglycemic hyperinsulinemic clamp studies in Asian Indian men without diabetes

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ABSTRACT

Aim: Fasting surrogate measures of insulin sensitivity are increasingly used in research and clinical practice. To assess the reliability of these measures, we aimed to evaluate multiple fasting surrogate measures simultaneously in non-diabetic subjects in comparison with the euglycemic hyperinsulinemic clamp study.
Methods: Sixteen normoglycemic male South Indian subjects were studied. After an overnight fast, blood samples were collected for glucose, insulin and lipid profile measurements, and stepped euglycemic hyperinsulinemic clamp studies were performed on all subjects. Steady state glucose infusion rates (M value) during low and high insulin phases of the clamp were calculated. Correlation of M value with surrogate markers of insulin sensitivity was performed. Predictive accuracy of surrogate indices was measured in terms of Root Mean Squared Error (RMSE) and leave-one-out cross-validation-type RMSE of prediction using a calibration model.
Results: M values showed a strong and significant correlation ($p < 0.01$) with the following surrogate markers: Fasting insulin ($r = -0.714$), Fasting glucose to insulin ratio (FGIR, $r = 0.747$) and Raynaud index ($r = 0.714$). FGIR had a significantly lower RMSE when compared with HOMA-IR and QUICKI.
Conclusions: Among the surrogate measures, FGIR had the strongest correlation with M values. FGIR was also the most accurate surrogate measure, as assessed by the calibration model.

1. Introduction

Communicable diseases, including diabetes mellitus, cardiovascular disease and stroke, are the major causes of mortality and morbidity in India (Sharma, 2013). Insulin resistance has been recognized as a critical factor in the evolution of these disorders (DeFronzo & Ferrannini, 1991). Insulin resistance is defined as an impaired response of glucose uptake and utilization to a known concentration of insulin when compared with the response in the normal population (Lebovitz, 2001). Measuring insulin resistance is of importance not only in research but also in clinical practice, playing a valuable role in the prevention and treatment of diseases such as Type 2 diabetes mellitus, coronary artery disease, stroke and polycystic ovary syndrome (PCOS) (Hanley, Williams, Stern, & Haffner, 2002; Kernan et al., 2003; Legro, Finegood, & Dunaif, 1998).

Over the past few decades, a number of methods have evolved in the assessment of insulin sensitivity (Muniyappa, Lee, Chen, & Quon, 2008). Amongst them, the euglycemic hyperinsulinemic clamp study (EHCS) is the gold standard (DeFronzo, Tobin, & Andres, 1979). EHCS is complex and expensive, making it difficult to use for epidemiological studies and clinical practice. Therefore, many alternate methods have been developed. Models that calculate insulin sensitivity from fasting glucose and fasting insulin values, such as the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and the Quantitative Insulin Sensitivity Check Index (QUICKI), are simple to perform and relatively inexpensive (Wallace & Matthews, 2002). However, they are recognized to have significant limitations in their ability to accurately quantify insulin resistance in comparison with the more rigorous insulin clamp studies (Muniyappa et al., 2008).

Conflicts of interest: none.

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Several surrogate measures of insulin sensitivity have been developed and shown to have varying degrees of correlation with the EHCS in Western populations. However, ethnic differences are known to affect the reliability of surrogate measures (Alvarez, Bush, Hunter, Brock, & Gower, 2008; Kang et al., 2005; Pisprasert, Ingram, Lopez-Davila, Munoz, & Garvey, 2013; Wallace, Levy, & Matthews, 2004). Factors such as degree of adiposity affect the accuracy of surrogate measures and may play a role in ethnic differences in reliability of those measures (Kim, Abbasi, & Reaven, 2004). Because fasting indices mainly reflect hepatic insulin sensitivity, ethnic differences in the site of insulin resistance, i.e. hepatic versus peripheral insulin resistance, can also affect the reliability of surrogate measures. Importantly, the Asian Indian population has a distinctive phenotype characterized by a higher prevalence of diabetes, increased adiposity, and larger waist circumference in proportion to the BMI when compared with Western populations. Therefore it is imperative to validate surrogate measures of insulin sensitivity among Indian subjects using the gold standard EHCS (Mohan, Sandeep, Deepa, Shah, & Varghese, 2007). Our group previously analyzed metabolic characteristics of normal subjects in India and found that the HOMA-IR appeared relatively low when compared with insulin sensitivity measured by M-values derived from the EHCS (Thomas et al., 2012). Muniyappa et al. (2010) also reported that surrogate indices of insulin sensitivity such as QUICKI and HOMA-IR had limited utility in the prediction of insulin sensitivity when compared with the EHCS in Asian-Indian men. Thus, studies identifying the most appropriate surrogate measure of insulin sensitivity for an Asian Indian population and other non-Western ethnic groups are clearly needed.

In this study, we aimed to find the most appropriate fasting surrogate measure of insulin sensitivity in a homogeneous group of non-diabetic male Indian subjects by comparing several surrogate measures against EHCS.

2. Materials and methods

The study protocol was approved by the Institutional Review Boards of the Christian Medical college, Vellore (Research Committee Minute No: 7722, 2012) and the Albert Einstein College of Medicine. The study included 16 healthy male South Indian subjects. Informed consent was obtained from all participants. The study was conducted at the Department of Endocrinology, Diabetes & Metabolism, Christian Medical College, Vellore. All subjects underwent detailed clinical examination and anthropometric measurements prior to inclusion. Fasting blood samples were taken for plasma glucose, serum insulin and lipid profile estimation.

Euglycemic hyperinsulinemic clamp studies were performed in all participants. Subjects reported to the Endocrinology Department clamp study room after an overnight fast. These 'pancreatic clamp' experiments consisted of 6 h of multiphasic soluble insulin infusion (Human Insulin Actrapid) with somatostatin (250 μg/h) infusion and replacement of basal glucoregulatory hormones (glucagon 1 ng/kg/min; growth hormone 3 ng/kg/min). Throughout the entire 6 h of study, the plasma glucose concentration was maintained at basal levels (~90 mg/dl) by a variable infusion of 20% Dextrose.

Basal phase: From $t = 0$ min to $t = 120$ min, optimal insulin infusion rates (IIRs) were selected in each individual by making frequent (~every 20–25 min) adjustments to IIRs in order to establish basal rates required to maintain euglycemia (90 mg/dl) without significant exogenous glucose infusion.

Low phase: Following establishment of basal insulin requirements, at $t = 120$ min the IIR was increased by 20 mU/m^2/min above basal, and was maintained at this rate for another 120 min. These rates were designed to optimally assess hepatic insulin sensitivity.

High phase: At $t = 240$ min, the IIR was increased to 80 mU/m^2/min, and maintained at that rate for the final 120 min of the study. These rates were designed to assess peripheral glucose uptake under maximal insulin stimulation.

To quantify glucose turnover, specifically rates of peripheral glucose disposal and endogenous glucose production, a primed continuous infusion of 6,6-glucose (D2G; Sigma-Aldrich) was initiated at $t = 0$ min and continued throughout the entire 6 h clamp study (initial bolus 200 mg/m^2 for 3 min, followed by 2 mg/min/m^2 continuous infusion for the entire study).

Rates of glucose appearance (Ra) and disappearance (Rd) and other indices of glucose turnover were estimated using Steele's (1959) equations. Endogenous glucose production (EGP) was determined by subtracting the rates of glucose infusion from the tracer-derived Ra.

Samples for D2G glucose determinations were obtained every 15 min. Plasma glucose was measured every 5 min to adjust the glucose infusion rate. Plasma glucose levels were measured using the glucose oxidase method (Analox GM9D; Analox Instruments, London, UK). Plasma insulin was measured by a chemiluminescent immunometric assay (% CV 7.3) (IMMULITE 2000 Insulin with IMMULITE 2000 Immunoassay System, Siemens healthcare Diagnostic products Ltd., Llanberis, Gwynedd, UK). Fasting plasma glucose for surrogate indices was measured by the glucose oxidase peroxidase method using reagents supplied by Roche, on Roche Modular P 800 system (% CV 3.6).

D2G determinations were performed at Albert Einstein College of Medicine. Plasma samples for Gas Chromatography–Mass Spectroscopy (GC–MS) were derivatized after protein precipitation to the aldonitrile pentacetate with hydroxylamine hydrochloride–acetic anhydride. GC/electron impact-mass spectrometry analysis was performed on an Agilent model 6890/5973 with a 7673 Agilent autosampler.

Insulin sensitivity was measured using the following formula during the final hour (steady state of plasma glucose concentration) of low and high insulin phases of the clamp:

$$M = \text{Glucose infusion rate–Space correction(SC)}$$

$$SC(\text{mg/kg/ min}) = (G_2 – G_1) * 0.0317$$

G_2 and G_1 are the plasma glucose concentrations (mg/dL) at the end and the beginning of the steady state phase of each step of the clamp.

M is a measure of whole body insulin sensitivity (DeFronzo et al., 1979). EGP is a measure of hepatic insulin sensitivity and Rd is a measure of peripheral insulin sensitivity.

Surrogate measures based on fasting glucose and insulin were considered for this study.

This study included a comprehensive list of previously published fasting surrogate indices. Appendix Table A.1 shows the list of indices and their formulae.

Normality of the data was determined using the Shapiro Wilk test. A correlation of surrogate markers of insulin sensitivity with the M value of low insulin phase, EGP and Rd of the clamp study was tested using the Spearman's rank correlation coefficient. A P value of <0.01 was considered statistically significant.

As correlation coefficients can sometimes be misleading, random calibration model analysis was performed as described in previous studies (Chen, Sullivan, & Quon, 2005; Muniyappa et al., 2010). Generalized linear models relating surrogate measures with the M value were derived. The square root of the mean squared error of prediction (RMSE) and Leave-one-out cross-validation-type root mean squared error of prediction (CVPE) were calculated for evaluating prediction accuracy of each surrogate measure. RMSE is estimated with the formula $[\sum e_i^2/(n-2)]^{1/2}$, where e_i is the difference between observed M value and the M value predicted by linear models of surrogate measures. CVPE is calculated as $[\sum e_{(i)}^2/n]^{1/2}$, where $e_{(i)}$ is estimated as the difference between the observed M value and the M value predicted by the model with the i^{th} subject excluded. To compare predictive accuracy among the surrogate measures, confidence intervals of pair wise differences in RMSE and CVPE of surrogate measures were done using a bootstrap percentile method, with 60,000 replications performed for each comparison.