Methods for Quantifying Insulin Resistance in Human Immunodeficiency Virus–Positive Patients

James W. Chu, Fahim Abbasi, George W. Beatty, Mandana Khalili, Johannes Koch, Alan Rosen, Jamey M. Schmidt, John D. Stansell, and Gerald M. Reaven

Various indirect indices have been used in human immunodeficiency virus (HIV)-infected individuals to assess insulin resistance, but the validity of these measures has not been rigorously assessed by comparison with physiologic methods of quantifying insulin-mediated glucose uptake (IMGU). We directly measured IMGU in 50 nondiabetic HIV-positive subjects by determining the steady-state plasma glucose (SSPG) concentration in response to a 3-hour continuous infusion of insulin, glucose, and somatostatin. Because steady-state plasma insulin concentrations were similar (~60 μU/mL) in all subjects, the SSPG concentrations provided direct assessments of insulin action. Relationships between SSPG levels and various surrogate measures of IMGU derived from the 75-g oral glucose tolerance test (OGTT) were determined. The indirect measure of IMGU most closely related to SSPG concentrations was the total integrated insulin response to a 75-g glucose load ($r = 0.78$, $P < .01$), accounting for approximately two thirds of the variability in SSPG ($r^2 = 0.61$). Other indirect measures of IMGU, including the homeostasis assessment for insulin resistance (HOMA-IR), were also significantly related to SSPG values, but had lower magnitudes of correlation ($r = 0.43$ to 0.61), thereby possessing limited ability to predict SSPG variability ($r^2 = 0.18$ to 0.37). In conclusion, indirect measures of IMGU need to be applied with caution when evaluating insulin action in HIV-infected patients.

TREATMENT OF human immunodeficiency virus (HIV) infection with antiretroviral therapy, including use of protease inhibitors (PI), has been linked to development of hypertriglyceridemia,[1-8] glucose intolerance,[4-9] and hyperinsulinemia.[6-8,10] Because such metabolic abnormalities are similar to those manifested in the insulin-resistance syndrome,[11] many studies,[4,6-8,12-14] but not all,[3,10,15] have suggested that PI use induces resistance to insulin-mediated glucose uptake (IMGU). However, with the exception of one study in HIV-negative volunteers,[14] reports of insulin resistance in PI-treated HIV-positive subjects have exclusively estimated IMGU by use of the homeostasis model assessment for insulin resistance (HOMA-IR)[3,4,7,10,12] or by analysis of other indirect indices of insulin action.[6,8,13,15]

In HIV-negative individuals, an extensive number of surrogate measures such as HOMA-IR have been previously proposed to predict IMGU.[16-21] These mathematical models are conceptually similar, derived from either fasting or postglucose-load concentrations of serum glucose and/or insulin. Although conveniently and advantageously applied in large-scale epidemiological studies, such indirect indices are inadequate representations of insulin action when applied to individuals, since IMGU is a physiologic parameter that can only be directly quantified during appropriate dynamic testing. Fasting measurements, which constitute the entire basis for the calculation of HOMA-IR[16] and Quantitative Insulin Sensitivity Check Index (QUICKI),[19,21] reflect the static end result of a host of metabolic processes,[22,23] some of which are unrelated to IMGU. Postglucose-load measurements, which are commonly applied in mathematical models to estimate insulin action,[17,18,20] are also insufficient for full evaluation of IMGU, since they result from a complex interplay between both insulin action and insulin secretion.[24,25] Therefore, without physiologic validation of these indirect indices of IMGU in HIV-positive subjects, it would be problematic to report on degrees of insulin action in this population. Previous studies in non–HIV-infected patients have already demonstrated potential pitfalls in using surrogate measures of IMGU to interpret differences in insulin resistance.[26-29]

The hyperinsulinemic euglycemic clamp method and the insulin suppression test are techniques for direct physiologic quantitation of insulin action, and the results from these two methods are highly correlated ($r > 0.90$).[30] The insulin suppression test determines a steady-state plasma glucose (SSPG) concentration as a measure of IMGU, and this assay has been previously validated by our group.[31] Studies comparing surrogate measures of IMGU to SSPG results in a cross-sectional study of 490 nondiabetic HIV-negative volunteers demonstrated that HOMA-IR and QUICKI each accounted for less than 40% of the variability in SSPG, that is, $r = 0.64$ and 0.60, respectively.[26,27] Thus, these indirect indices of insulin resistance appear to be of limited utility in HIV-negative individuals. In this current study, we sought to similarly define the relationships between indirect and direct measures of insulin action in a cohort of HIV-positive subjects. These comparisons would help evaluate the validity of applying indirect indices of IMGU in studying HIV-positive patients, given the multiple previous reports that utilized HOMA-IR and other surrogate measures to conclude that PI therapy causes insulin resistance.

MATERIALS AND METHODS

The study population consisted of 50 volunteers (47 men, 3 women) with confirmed HIV infection and no previous diagnosis of diabetes mellitus. Subjects were recruited from the University of California San

From the Department of Medicine, Stanford University School of Medicine, Stanford, CA; and the Department of Medicine, University of California San Francisco School of Medicine, San Francisco, CA.

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Address reprint requests to Gerald M. Reaven, MD, CVRB Second Floor South, Stanford University Medical Center, Stanford, CA 94305-5406.

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Table 1. Fasting Metabolic Characteristics and Indirect Indices of Insulin Action in 50 HIV-Positive Subjects

Measures	Mean ± SEM (Range)
$Glucose_0$ (mg/dL)	93 ± 1 (75-115)
Glucose AUC (mg/dL)	357 ± 9 (252-561)
$Insulin_0$ (μU/mL)	11.7 ± 1.3 (1.2-39.7)
Insulin AUC (μU/mL)	150 ± 13 (43-393)
FFA_0 (μEq/L)	512 ± 58 (83-2132)
FFA AUC (μEq/L)	1135 ± 130 (241-4882)
$Glucose_0/insulin_0$ (mg/dL · [μU/mL]$^{-1}$)	13.5 ± 1.8 (2.5-79.1)
HOMA-IR	2.8 ± 0.3 (0.3-10.5)
QUICKI	0.35 ± 0.01 (0.28-0.49)
QUICKI-FFA	0.41 ± 0.01 (0.26-0.72)
Stumvoll index	0.118 ± 0.002 (0.090-0.145)
SSPG (mg/dL)	147 ± 10 (45-308)

Abbreviations: AUC, total integrated area under the curve after a 75-g glucose load; FFA, free fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; QUICKI-FFA, revised quantitative insulin sensitivity check index, with use of free fatty acids; SSPG, steady-state plasma glucose concentration.

Francisco (UCSF) Positive Health Program at San Francisco General Hospital (SFGH). Each volunteer provided written informed consent prior to enrollment in a study approved by the UCSF Committee on Human Research. Individuals with advanced liver disease, active opportunistic infections, and malignancies were excluded, as were those taking anabolic and antihyperglycemic medications. Additional exclusion criteria included prothrombin time greater than 2.0 seconds prolonged, international normalized ratio greater than 1.5, total bilirubin greater than 2.0 mg/dL, serum albumin less than 3.0 g/dL, creatinine greater than 2.0, absolute neutrophil count less than 500 cells/μL, uncontrolled diarrhea, weight change greater than 5 kg in the preceding 3 months, prior treatment for viral hepatitis, and active substance abuse.

Enrolled participants had a mean (± SEM) age of 42 ± 1 years (range, 25 to 62), and body mass index (BMI) of 25.6 ± 0.5 kg/m^2 (range, 18.3 to 34.8). The median CD4 count was 374 cells/μL, and the median HIV RNA load was 85,000 copies/mL. Twenty-three subjects were taking PI therapy, 14 were on other antiretroviral therapy, and 13 were taking no anti-HIV medications at the time of the study. Patients on HIV-1 antiretroviral regimens were maintained on the same treatment for at least 12 weeks prior to participation. None of the subjects had acquired immunodeficiency syndrome (AIDS)-defining illnesses, and none experienced wasting or cachexia. No subjects were using glucocorticoids, megesterol acetate, growth hormone, or pentamidine at the time of the study. Cross-sectional comparisons of metabolic measures between individuals taking and not taking PI therapy have been reported elsewhere (Beatty et al, submitted for publication).

Subjects were admitted to the SFGH General Clinical Research Center (GCRC) for all research measurements. Participants underwent routine medical interview, anthropometrical measurements, and physical examination. A 75-g oral glucose tolerance test (OGTT) was performed in the GCRC after an overnight fast of 12 hours. Blood samples were taken for measurements of plasma glucose,[32] insulin,[33] and free fatty acids[34] at 0, 30, 60, 120, and 180 minutes after the oral ingestion of a 75-g glucose challenge. The total integrated glucose and insulin responses were quantified separately by calculating the respective areas under the curve, using the trapezoidal method. Seven subjects (14%) were assigned putative diagnoses of impaired glucose tolerance, as determined by 120-minute postload glucose concentrations ranging between 140 and 199 mg/dL.

On the same admission to the GCRC, after another overnight fast of 12 hours, each subject underwent the insulin-suppression test, as previously introduced and validated by our research group.[30,31,35] Intravenous catheters were placed into each of the antecubital fossa, one used for the administration of a 180-minute infusion of somatostatin (250 μg/h), insulin (25 mU · m^{-2} · min^{-1}) and glucose (240 mg · m^{-2} · min^{-1}), and the other for collection of timed blood samples every 30 minutes initially, and then at 10-minute intervals from 150 to 180 minutes of the infusion. These last 4 time points permit the determination of the steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations for each individual. Because SSPI concentrations are similar between subjects, the SSPG concentration provides a direct measure of the ability of insulin to mediate disposal of an infused glucose load; higher values of SSPG denote increasing degrees of insulin resistance.

HOMA-IR was calculated using the formula proposed by Matthews et al (HOMA-IR = $insulin_0 \cdot glucose_0$), with insulin expressed in μU/mL and glucose in mmol/L.[16] QUICKI and QUICKI-FFA were calculated using the formulae proposed by Katz et al (QUICKI = $1/[\log(insulin_0) + \log(glucose_0)]$),[19] and Perseghin et al (QUICKI-FFA = $1/[\log(insulin_0) + \log(glucose_0) + \log(\text{free fatty acids}_0)]$),[21] respectively, with insulin expressed in μU/mL, glucose in mg/dL, and free fatty acids in mmol/L. The Stumvoll index was derived from the formula proposed by Stumvoll et al (Stumvoll index = $0.222 - [0.00333 \cdot BMI] - [0.0000779 \cdot Ins_{120\ min}] - [0.000422 \cdot age]$), with insulin expressed in pmol/L, BMI in kg/m^2, and age in years.[18] The Matsuda index[17] was not calculable, because this measure incorporates a nonstandard 90-minute time point in the OGTT. All analyses were performed using the Systat 9.0 package for Windows. Indirect indices of insulin resistance were correlated to SSPG results by calculation of the Pearson correlation coefficient. Statistical significance was assigned at $P < .05$.

RESULTS

Table 1 shows the metabolic characteristics of the 50 HIV-positive patients, including fasting and integrated post-load concentrations of plasma glucose, insulin, and FFA. Calculated indirect indices of IMGU are also given. Table 2 depicts the Pearson correlation coefficients between SSPG and each of these measured and derived parameters, as determined for the entire group, and separately for the 27 patients not on PI

Table 2. Univariate Correlation Coefficients Between SSPG Concentrations and Various Fasting and Calculated Parameters

Indirect Measures of Insulin Action	Entire Cohort (N = 50)	Non-PI (n = 27)	PI (n = 23)
$Glucose_0$	0.33	0.26*	0.42
Glucose AUC	0.52	0.45	0.69
$Insulin_0$	0.61	0.61	0.68
Insulin AUC	0.78	0.76	0.88
FFA_0	0.35	0.47	0.34*
FFA AUC	0.12*	0.48	0.14*
$Glucose_0/insulin_0$	−0.43	−0.45	−0.46
HOMA-IR	0.61	0.61	0.68
QUICKI	−0.56	−0.52	−0.60
QUICKI-FFA	−0.64	−0.61	−0.68
Stumvoll index	−0.43	−0.37*	−0.49

*$P > .05$. All other correlation coefficients were statistically significant, with $P < .05$.

Abbreviations: AUC, total integrated area under the curve after a 75-g glucose load; FFA, free fatty-acids; HOMA-IR, homeostasis model assessment of insulin resistance; PI, protease inhibitor use; QUICKI, quantitative insulin sensitivity check index; QUICKI-FFA, revised quantitative insulin sensitivity check index, with use of free fatty acids; SSPG, steady-state plasma glucose concentration.

therapy, and the 23 patients on PI therapy. Regarding the correlation coefficients calculated for analysis of the entire cohort, all parameters demonstrated significant relationships with SSPG concentrations (magnitude of $r = 0.33$ to 0.78, $P < .05$) except for the total integrated FFA response to the 75-g glucose load. The total integrated insulin response to a 75-g glucose challenge manifested the tightest association with SSPG levels ($r = 0.78$, $r^2 = 0.61$, $P < .01$).

With respect to the relationships between each parameter and SSPG in the PI-treated subgroup, the magnitude of each correlation coefficient was slightly greater than each corresponding one in the non PI-treated subgroup ($r = 0.42$ to 0.88 *v* $r = 0.26$ to 0.76), with the exception of the two correlations involving fasting FFA and integrated postglucose FFA response. Despite these differences between the treatment subgroups, the total integrated insulin response to a 75-g glucose challenge still demonstrated the tightest association with SSPG levels in both groups ($r = 0.88$ for PI group, $r = 0.76$ for non-PI group, $P < .01$ for both).

DISCUSSION

Previous studies have implicated PI therapy in the development of insulin resistance, based on determinations of HOMA-IR and other indirect indices of IMGU. However, the application of these surrogate measures of insulin action has never been validated in HIV-positive individuals. Our results show that HOMA-IR correlates with SSPG ($r = 0.61$, $P < .05$), but that HOMA-IR accounts for approximately only one-third of the variability in IMGU ($r^2 = 0.37$). Furthermore, we observed that the strength of the association between HOMA-IR and SSPG was virtually indistinguishable from that of the correlation between fasting insulin and SSPG ($r = 0.61$, $P < .05$). Univariate correlations between SSPG and other indirect measures of IMGU showed that the best predictor of insulin action was the total integrated insulin response to a 75-g glucose load ($r = 0.78$, $P < .01$). However, this result suggested that even the most useful surrogate measure of insulin action could account for less than two thirds of the variability in IMGU ($r^2 = 0.61$).

These findings of statistically significant but numerically modest correlations between SSPG and the indirect measures of insulin action are remarkably consistent with previous results from a study of 490 nondiabetic HIV-negative individuals.[26] We observed in this earlier report that SSPG was modestly correlated to HOMA-IR ($r = 0.62$), fasting insulin ($r = 0.61$), and total integrated insulin response to a 75-g glucose load ($r = 0.67$), with the latter measure achieving the strongest relationship with SSPG.[26] Thus, our current results reiterate the previous findings in demonstrating that HOMA-IR does not confer much additional benefit over fasting insulin concentrations to predict IMGU, and that neither can account for more than 40% in the variability of SSPG levels.

Our confidence in using the insulin suppression test to quantify insulin resistance is supported by extensive experience applying SSPG results in a variety of clinical situations. We have previously demonstrated that estimates of IMGU using the hyperinsulinemic, euglycemic clamp, often considered the "gold standard," were highly correlated with SSPG concentrations in normal subjects and patients with type 2 diabetes mellitus.[30] In addition, in a number of cross-sectional studies involving subjects with high SSPG concentrations, we have demonstrated strong associations between insulin resistance and various metabolic and vascular abnormalities, including hypertension, impaired glucose tolerance and/or type 2 diabetes mellitus, hypertriglyceridemia, low high-density lipoprotein (HDL)-cholesterol,[11] small dense low-density lipoprotein (LDL) particles,[36] increased postprandial lipemia,[37] high levels of plasminogen activator inhibitor-1,[38] and elevated concentrations of intercellular adhesion molecules,[39] all of which may portend an elevated risk for future atherosclerotic disease. Furthermore, when we applied SSPG results to stratify longitudinal cohorts, the most insulin-resistant individuals were demonstrated to have significantly greater long-term risks of developing type 2 diabetes mellitus and cardiovascular disease.[40,41]

In summary, our results suggest that considerable caution must be undertaken when using indirect indices of insulin resistance to determine insulin resistance in HIV-infected individuals. The modest correlation between an indirect measure (HOMA-IR) and a direct quantitation (insulin suppression test) of IMGU suggests that fasting concentrations of plasma glucose and insulin, both of which are used in the HOMA-IR calculation, are significantly influenced by metabolic factors other than insulin-mediated glucose uptake. Our results also suggest that the application of other indirect indices of IMGU, including QUICKI, QUICKI-FFA, the Stumvoll index, and the fasting glucose:insulin ratio, is of limited utility in predicting insulin action in HIV-positive individuals. The insulin response to a 75-g glucose load appeared to yield the strongest association with SSPG, thus potentially providing the most useful surrogate marker of IMGU.

Although readily available indirect indices of IMGU may be usefully applied in large-scale epidemiological studies to stratify groups by degrees of insulin resistance, any determinations of insulin action using these methods in small-sampled selective cohorts may not be valid. Our results demonstrate the limited ability of HOMA-IR and various indirect indices to accurately assess insulin resistance in 50 nondiabetic HIV-positive individuals. The poor predictive value of such surrogate measures of IMGU may account for the discordance in observations reported by various authors on the issue of whether PI therapy induces insulin resistance in HIV-positive subjects.[3,4,6-8,10,12-15] Therefore, we suggest that future studies of insulin resistance in HIV-infected patients should utilize validated, direct, physiologic methods of quantifying insulin action.

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