Obesity, ectopic lipids, and insulin resistance

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CHAPTER 3

Methods for quantifying adipose tissue insulin resistance in overweight/obese humans


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ABSTRACT

Background/Objectives
Insulin resistance of adipose tissue is an important feature of obesity-related metabolic disease. However, assessment of lipolysis in humans requires labor-intensive and expensive methods, and there is limited validation of simplified measurement methods. We aimed to validate simplified methods for the quantification of adipose tissue insulin resistance against the assessment of insulin sensitivity of lipolysis suppression during hyperinsulinemic-euglycemic clamp studies.

Subjects/Methods
We assessed the insulin-mediated suppression of lipolysis by tracer-dilution of \[^{1,1,2,3,3-2H_5}\]glycerol during hyperinsulinemic-euglycemic clamp studies in 125 overweight or obese adults [85 men, 40 women; age 50±11 years; body mass index (BMI) 38±7 kg/m\(^2\)]. Seven indices of adipose tissue insulin resistance were validated against the reference measurement method.

Results
Low-dose insulin infusion resulted in suppression of the glycerol rate of appearance (Ra) ranging from 4% (most resistant) to 85% (most sensitive), indicating a good range of adipose tissue insulin sensitivity in the study population. The reference method correlated with i) insulin-mediated suppression of plasma glycerol concentrations (r=0.960, p<0.001), ii) suppression of plasma free fatty acid (FFA) concentrations (r=0.90, p<0.001), iii) the Adipo-IR index (fasting plasma insulin-FFA product; r=-0.53, p<0.001), iv) the fasting plasma insulin-glycerol product (r=-0.47, p<0.001), v) the ATIRI index (fasting plasma insulin-basal lipolysis product; r=0.46, p<0.001), vi) the QUICKI-FFA index (r=0.62, p<0.001), and vii) the QUICKI-glycerol index (r=0.67, p<0.001). Bland-Altman plots showed no systematic errors for the suppression indices, but proportional errors for all fasting indices. Receiver-operator characteristic (ROC) curves confirmed that all indices were able to detect adipose tissue insulin resistance (AUC≥0.801, p<0.001).

Conclusions
Adipose tissue insulin sensitivity (that is, the antilipolytic action of insulin) can be reliably quantified in overweight and obese humans by simplified index methods. The sensitivity and specificity of the Adipo-IR index and the fasting plasma insulin-glycerol product, combined with their simplicity and acceptable agreement, suggest that these may be most useful in clinical practice.
INTRODUCTION

Lipolysis of stored triglycerides in adipose tissue, resulting in the release of FFA and glycerol into the plasma, is a key metabolic process for mobilization of endogenous energy substrates under fasting conditions [156]. Adipose tissue lipolysis is enhanced by glucagon, catecholamines, and growth hormone [77,157], and suppressed by insulin [158]. Insulin resistance of adipose tissue, defined as a diminished antilipolytic effect of insulin, is an important feature of obesity-related metabolic disease [76], resulting in increased (and inappropriate) release of FFA into the plasma. This, in turn, may lead to lipotoxicity in multiple organs: an elevated FFA flux contributes to the development of steatosis in the liver [159] and accumulation of intramyocellular lipids [160,161].

The current gold standard for quantification of insulin resistance in adipose tissue in humans is the determination of lipolysis fluxes by tracer-dilution techniques during continuous intravenous insulin infusion [162]. In this regard, both whole-body glycerol and FFA release into the circulation, measured using stable isotope-labeled glycerol or FFA tracers, have been validated to (qualitatively) reflect adipose tissue lipolysis under most circumstances [163]. However, lipolysis-derived fatty acids may be re-esterified within adipocytes, whereas lipolysis-derived glycerol is almost completely released because adipocyte glycerol kinase activity is low [164]. Therefore, the rate of glycerol release into the circulation provides a quantitatively good reflection of whole-body adipose tissue lipolytic activity/complete triglyceride hydrolysis under fasting or stimulated conditions [162,163,165-168]. Unfortunately, these tracer studies are expensive and labor-intensive and, therefore, unfeasible in large trials or clinical settings. To date, multiple indices to estimate resistance to the glucoregulatory effects of insulin, such as the homeostasis model assessment of insulin resistance (HOMA-IR) [137] or Matsuda index [47], have been developed and validated (reviewed in [39,169,170]). In contrast, although several indices for the estimation of insulin resistance of adipose tissue have been proposed in literature [171-174], data on quantification of adipose tissue insulin resistance in humans is still scarce due to limited validation of these simplified methods [171,174,175].

In the present study, we aimed to validate simplified methods for the quantification of adipose tissue insulin resistance against the gold standard measurement method (that is, measurement of the Ra of glycerol using [1,1,2,3,3-2H5]glycerol infusion during hyperinsulinemic-euglycemic clamp conditions) in a large cohort of overweight and obese subjects.

SUBJECTS AND METHODS

Subjects participated in different metabolic studies at the Academic Medical Center (Amsterdam, The Netherlands). Overweight (BMI >25 kg/m²) or obese (BMI >30 kg/m²) subjects were recruited from the general population through local advertisements and the outpatient clinics of 3 obesity centers in The Netherlands. Consecutive subjects were eligible for the present study if they underwent a hyperinsulinemic-euglycemic clamp, with continuous [1,1,2,3,3-2H5]glycerol infusion to assess the rate of lipolysis, according
to standard operating procedures. Subjects, who were studied after an intervention or during a non-standard clamp protocol (for instance, different rate or duration of insulin infusion), were excluded. Other exclusion criteria were substance abuse (alcohol >2 units/day, recreational drugs), use of exogenous insulin, incretin mimetics, antipsychotics, or antidepressants, or any somatic disorder except for obesity-related conditions (that is, dyslipidemia, hypertension, or obstructive sleep apnea). All subjects completed a medical evaluation, including history, physical examination, and blood tests, before the study visit. All procedures were approved by the Academic Medical Center medical ethics committee, and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

**Experimental protocol**

Insulin sensitivity of adipose tissue was determined during a hyperinsulinemic-euglycemic clamp study, with primed continuous infusion of [1,1,2,3,3-2H5]glycerol, as previously described [130]. Subjects were studied after an overnight fast. A catheter was inserted into a forearm vein to infuse glucose, insulin, and the glycerol tracer. Another catheter was inserted into a contralateral forearm vein to obtain blood samples. Blood samples were obtained before the start of tracer infusion to determine background tracer enrichment. At 0800 h (t=-2 h), a primed continuous infusion of [1,1,2,3,3-2H5] glycerol (>99% enriched; Cambridge Isotopes, Andover, MA, USA; prime 1.6 μmol/kg, continuous 0.11 μmol·kg⁻¹·min⁻¹) was started and continued until the end of the experiment. After 2 h of tracer equilibration, at 1000 h (t=0 h), infusion of insulin (Actrapid; Novo Nordisk Farma, Alphen aan de Rijn, The Netherlands) was started at a rate of 20 mU·m⁻²·min⁻¹ and continued for 130 min. During insulin infusion, plasma glucose was measured every 10 min and 20% glucose was infused at a variable rate to maintain plasma glucose at 5.0 mmol/l. At t=0 and t=2 h, 3 blood samples with 5-min intervals were drawn to assess plasma tracer enrichment and plasma concentrations of insulin, FFA, and glycerol.

Plasma glucose, insulin, and FFA were determined as described [176]. Plasma glycerol concentrations and enrichment of [1,1,2,3,3-2H₅]glycerol (tracer-to-tracee ratio) were determined by gas chromatography–mass spectrometry as described [136].

**Calculations**

The Ra of glycerol was calculated from 3 steady-state plasma glycerol enrichment measurements using a modified version of the Steele’s equation for the steady state as described [40]. Adipose tissue insulin sensitivity was defined as the suppression of glycerol Ra by low-dose insulin infusion (relative to basal Ra) and adjusted for the steady-state plasma insulin concentration (that is, percentage suppression of glycerol Ra / insulin concentration during the clamp) [173,175].

The rate of lipolysis in adipose tissue is the major determinant of plasma FFA and glycerol concentrations [177]. Therefore, we first assessed the insulin-mediated suppression of circulating FFA and glycerol concentrations (both also adjusted for the steady-state plasma insulin concentration during the clamp) as markers of adipose tissue insulin
Quantifying adipose tissue insulin resistance

sensitivity [178]. In addition, we identified several indices for the estimation of adipose tissue insulin resistance from the literature. The Adipose tissue Insulin Resistance (Adipo-IR) index is based on the notion that the Ra of FFA and plasma FFA concentrations are correlated and calculated as fasting plasma insulin x fasting plasma FFA concentrations [171,172,179,180]. Another index is calculated as fasting plasma insulin x fasting plasma glycerol concentrations [174]. The Adipose Tissue Insulin Resistance Index (ATI-RI) is calculated as fasting plasma insulin concentration x basal rate of lipolysis [173]. The modified Quantitative Insulin Sensitivity Check Index (revised QUICKI) incorporates fasting plasma FFA concentrations into the original QUICKI and is calculated as 1 / (log glucose + log insulin + log FFA) [181]. Finally, the QUICKI-glycerol is calculated in the same way as the revised QUICKI, but replaces log FFA with log fasting plasma glycerol [182]. Thus, a total of 7 methods (indices) for the estimation of adipose tissue insulin sensitivity were calculated.

Statistical analysis
Data were tested for normality by inspection of histograms. Unless stated otherwise, normally distributed continuous variables are presented as mean ± standard deviation (SD) and non-normally distributed data as median [interquartile range (IQR)]. Pearson’s (r) correlation coefficients were used to evaluate correlations between 2 normally distributed parameters. We performed multiple linear regression analysis to evaluate the effects of confounding factors on, and define models for, the prediction of adipose tissue insulin sensitivity from index methods. Here, non-normally distributed data entered into the model were first log-transformed. We used Bland-Altman plots and computed regression-based 95% limits of agreement (LoA) to analyze bias and concordance between each index method and the reference method [183]. To compare index and standard on the same scale of measurement (and construct Bland-Altman plots), we predicted adipose tissue insulin sensitivity from each method using the prediction model and then compared predicted vs observed values. ROC curves were used to determine the diagnostic accuracy of index methods for the identification of severe adipose tissue insulin resistance, which we here defined as the lowest quartile of adipose tissue insulin sensitivity in the study cohort [184]. A p-value <0.05 was considered significant. Analyses were performed using IBM SPSS Statistics v23 (Armonk, NY, USA) and GraphPad Prism v6 (La Jolla, CA, USA).
RESULTS

We included 125 overweight or obese participants (Table 3.1). They had elevated fasting plasma insulin concentrations and HOMA-IR, reflecting insulin resistance at baseline [184]. Two subjects had pre-existing non-insulin-dependent type 2 diabetes and were treated with oral hypoglycemic agents. Two additional subjects had fasting plasma glucose concentrations >7.0 mmol/l on the morning of the clamp [185].

Table 3.1. Baseline characteristics of included subjects (n=125).

<table>
<thead>
<tr>
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<th>Baseline characteristics of included subjects (n=125).</th>
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<tbody>
<tr>
<td></td>
<td>Male sex (%) 85 (68)</td>
</tr>
<tr>
<td></td>
<td>Age (years) 50±11</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²) 38±7</td>
</tr>
<tr>
<td></td>
<td>Triglycerides (mmol/l) 1.4 (1.0-2.0)</td>
</tr>
<tr>
<td></td>
<td>Total cholesterol (mmol/l) 5.0±1.0</td>
</tr>
<tr>
<td></td>
<td>LDL (mmol/l) 3.1±0.9</td>
</tr>
<tr>
<td></td>
<td>HDL (mmol/l) 1.1±0.3</td>
</tr>
<tr>
<td></td>
<td>ALT (U/l) 31 (23-39)</td>
</tr>
<tr>
<td></td>
<td>Glucose (mmol/l) 5.3±0.7</td>
</tr>
<tr>
<td></td>
<td>Insulin (pmol/l) 114±74</td>
</tr>
<tr>
<td></td>
<td>HOMA-IR 4.5±3.0</td>
</tr>
</tbody>
</table>

Data are count (%), mean ± SD, or median (IQR).

Insulin infusion at a rate of 20 mU·m⁻²·min⁻¹ during the clamp suppressed plasma FFA concentrations by 79±12%, plasma glycerol concentrations by 59% (50-68), and the glycerol Ra by 60% (51-68) (Table 3.2). Insulin-mediated suppression of glycerol Ra ranged from 4% (most resistant) to 85% (most sensitive), demonstrating that the insulin infusion dose of our experimental protocol was adequate to detect differences in adipose tissue insulin sensitivity in the present cohort of overweight and obese subjects (Supplemental Figure S1). Isotopic steady state was confirmed during the last 10 min of the clamp (Supplemental Table S1).

Table 3.2. Data from hyperinsulinemic-euglycemic clamp studies (n=125).

<table>
<thead>
<tr>
<th></th>
<th>Basal state</th>
<th>Hyperinsulinemic clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute values</td>
<td>Relative to basal</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>114±74</td>
<td>316±109</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.62±0.16</td>
<td>0.13±0.08</td>
</tr>
<tr>
<td>Glycerol (µmol/l)</td>
<td>79 (63-108)</td>
<td>30 (23-52)</td>
</tr>
<tr>
<td>Glycerol Ra (µmol·kg⁻¹·min⁻¹)</td>
<td>2.2 (1.9-2.9)</td>
<td>1.0 (0.7-1.3)</td>
</tr>
</tbody>
</table>

Data are mean ± SD or median (IQR).
We next determined whether we could predict tracer-determined adipose tissue insulin sensitivity from several simplified methods or indices. Notably, all tested indices correlated with reference method-measured adipose tissue insulin action in univariate analyses (Figures 3.1A-G). Insulin-mediated suppressions of plasma glycerol and FFA concentrations showed particularly strong correlations (Figures 3.1A-B), but the fasting blood sample-derived indices also correlated reasonably well with tracer-determined adipose tissue insulin sensitivity (Figures 3.1C-G). Multiple regression analyses showed that neither sex nor BMI significantly affected the prediction of adipose tissue insulin sensitivity from any of the tested indices, except for suppression of FFA. Sex, but not BMI, was an independent factor in the prediction of adipose tissue insulin sensitivity from insulin-mediated suppression of FFA. Therefore, for this index, we present regression formulas for men and women separately (Supplemental Table S2). For the other indices, neither sex nor BMI were significant factors and thus not included in the prediction models (Supplemental Table S2).

In addition to the classic correlation analysis, we used 2 other methods to compare each index to the reference. First, we constructed Bland-Altman plots to evaluate the agreement between measurements [183]. As shown in Figures 3.2A-B, no systematic bias was apparent for comparisons between the suppressions of plasma glycerol or FFA concentrations vs tracer-determined adipose tissue insulin sensitivity. The fasting blood sample-derived indices all showed proportional biases compared to the reference method (Figures 3.2C-G); these plots revealed that fasting indices underestimated adipose tissue insulin sensitivity at high values (sensitive), whereas they overestimated it at low values (resistant). The range within which most differences between 2 methods will fall is defined by the 95% LoA, which are also shown in Figures 3.2A-G. Overall, agreement was best for the 2 suppression indices, acceptable for Adipo-IR, fasting insulin x fasting glycerol, and ATIRI, and poor for the 2 QUICKI-based indices. Discrepancies between index and reference methods seemed to mostly appear in the lower range of sensitivity.

Finally, to test the predictive power of simplified methods, we constructed ROC curves for the detection of adipose tissue insulin sensitivity below the lowest quartile (Supplemental Figure S2). As demonstrated by the areas under the ROC curve (aROC) (Table 3.3), the 2 suppression indices had good diagnostic accuracy and the fasting indices had acceptable diagnostic accuracy for the discrimination between adipose tissue insulin sensitivity and resistance in the present cohort. At the best cutoff point of the curve, sensitivity of these tests ranged from 67-97% and specificity ranged from 55-90% (Table 3.3). Given the 25% prevalence of adipose tissue insulin resistance in this analysis, it follows that all indices had negative predictive values ≥89%, whereas positive predictive values ranged from 42% (QUICKI-glycerol) to 76% (suppression of glycerol).
Figure 3.1. Correlations between isotope-labeled tracer measures of adipose tissue insulin sensitivity (on the y-axes) and (A) insulin-mediated suppression of plasma glycerol concentrations, (B) insulin-mediated suppression of plasma FFA concentrations, (C) the Adipo-IR, (D) the product of fasting plasma insulin and fasting glycerol concentrations, (E) the ATIRI, (F) the revised QUICKI, and (G) the QUICKI-glycerol. Men (●) and women (○) are indicated. Parameters that were assessed during hyperinsulinemic-euglycemic clamp conditions (that is, suppression of glycerol Ra, suppression of glycerol, and suppression of FFA) were adjusted for the steady-state insulin concentration.
Figure 3.2. Bland-Altman plots showing the agreement between adipose tissue insulin sensitivity measured by tracer method (observed) and by index methods (predicted). The mean difference (solid line) indicates whether there is a systematic error and the 95% LoA (dashed line) indicate the range within which most differences between 2 methods will fall.
DISCUSSION

Data from the present study demonstrate that the insulin-induced suppressions of plasma glycerol and FFA concentrations are accurate methods for evaluating adipose tissue insulin sensitivity in overweight and obese adults. Thus, adipose tissue insulin sensitivity can be quantified with good validity in a large cohort without the necessity to use expensive and complex stable isotope-labeled metabolic tracer methods. In addition, the data show that adipose tissue insulin resistance can be estimated from even simpler methods. Both the Adipo-IR and fasting insulin x fasting glycerol indices are determined in the basal state and do not require infusion of exogenous insulin for their assessment. Although the precision of these fasting indices is lower than the precision of the dynamic (hyperinsulinemic suppression) indices, the Adipo-IR and fasting insulin x fasting glycerol are still reasonably good quantifications of adipose tissue insulin sensitivity in overweight and obese adults, and their simplicity may promote the widespread application in clinical and/or research settings.

Table 3.3. Accuracy of tests for the identification of severe adipose tissue insulin resistance.

<table>
<thead>
<tr>
<th>Index</th>
<th>aROC</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppression of glycerol / insulin</td>
<td>0.962</td>
<td>&lt;0.152</td>
<td>97%</td>
<td>90%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Suppression of FFA / insulin</td>
<td>0.927</td>
<td>&lt;0.211</td>
<td>87%</td>
<td>86%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adipo-IR</td>
<td>0.811</td>
<td>&gt;75</td>
<td>81%</td>
<td>73%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting insulin x fasting glycerol</td>
<td>0.822</td>
<td>&gt;12330</td>
<td>67%</td>
<td>86%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ATIRI</td>
<td>0.823</td>
<td>&gt;205</td>
<td>97%</td>
<td>55%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Revised QUICKI</td>
<td>0.801</td>
<td>&lt;0.551</td>
<td>74%</td>
<td>75%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QUICKI-glycerol</td>
<td>0.811</td>
<td>&lt;0.262</td>
<td>90%</td>
<td>59%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Adipose tissue insulin resistance was defined as the lowest quartile of tracer-determined adipose tissue insulin sensitivity. Optimal cutoff points maximize sensitivity + specificity and were determined using ROC curves analysis.

Good correlation does not necessarily mean that there also is good agreement between 2 measurement methods [183]. Therefore, we used 2 additional methods to compare the indices to clamp-derived adipose tissue insulin sensitivity. Bland-Altman plots are designed to evaluate systematic errors and agreement between 2 methods. There is currently no consensus regarding the interpretation of agreement in method-comparison studies; this should be based on clinical judgment. If the 95% LoA are clinically acceptable, then 2 methods can be used interchangeably [183]. In this study, Bland-Altman analyses indicated acceptable concordance between the 2 suppression indices and the clamp, because there were no systematic errors and the 95% LoA were small. Keeping the normal within-person day-to-day variation in clamp results or FFA kinetics in mind [186-188], we also considered the agreement between the clamp and the Adipo-IR, fasting insulin x fasting glycerol, or ATIRI indices to be clinically acceptable. In fact, the precision of these indices is comparable to that of commonly used glucoreg-
ulatory surrogate indices including HOMA-IR [189,190]. The accuracy of these tests to
detect adipose tissue insulin resistance was confirmed by ROC curves analysis. Here, we
were able to discriminate between adipose tissue insulin sensitivity and resistance using
all tested indices. However, the sensitivity and specificity of Adipo-IR and fasting insulin
x fasting glycerol, combined with their simplicity and acceptable agreement with the
clamp, suggest that these may be most useful in clinical practice. Importantly, Bland-Alt-
man analyses also revealed that all fasting blood sample-derived indices proportionally
underestimated high adipose tissue insulin sensitivity and overestimated low adipose
tissue insulin sensitivity. This should be taken into account when interpreting estima-
tions of adipose tissue insulin sensitivity derived from these indices.

Adipose tissue insulin resistance is associated with many of the metabolic complica-
tions of obesity [191]. Given the current obesity epidemic [192], adipose tissue insulin
resistance likely contributes to an immense global health burden [193]. It is essential to
elucidate the pathogenesis of adipose tissue insulin resistance, and the mechanisms
that lead to the development of obesity-related metabolic complications [194]. There-
fore, precise and reproducible quantification of adipose tissue insulin sensitivity is im-
portant, and the validation of simplified measurement methods is highly relevant [175].

Our results are in line with previous, albeit limited, human validation studies [173,174].
The product of fasting insulin x fasting glycerol accurately predicted the dynamic in-
sulin-mediated suppression of plasma glycerol concentrations in 29 postmenopausal
overweight or obese, but otherwise healthy women [174]. This index, however, was
never validated against tracer-determined adipose tissue insulin sensitivity. The Adi-
po-IR, theoretically devised on the basis of observations that FFA turnover and plas-
ma FFA concentrations are correlated under continuous insulin infusion conditions
[171,172,179,180], has not yet been validated against gold standard-measured adipose
tissue insulin sensitivity [175]. Since this index has been used in several studies to date
[178,195,196], validation of this method in the present study is especially relevant. The
ATIRI correlated with adipose tissue insulin sensitivity, assessed using the hyperinsuline-
mic-euglycemic clamp method with infusion of a palmitate tracer, in 47 non-diabetic
obese adults with varying glucose tolerance (Spearman's rho=-0.85, p<0.001) [173].
Although the correlation we found was slightly weaker (Pearson's r=-0.46, p<0.001), we
now independently confirm that the ATIRI predicts adipose tissue insulin sensitivity dur-
ing hyperinsulinemic conditions. Nevertheless, this index provides few advantages as it
still requires the use of a stable isotope-labeled tracer for the assessment of the basal
lipolysis flux. The revised QUICKI and QUICKI-glycerol both correlate well with insulin's
ability to stimulate glucose disposal during a clamp [181,182], but - to our knowledge
- have not been related to insulin's ability to suppress lipolysis. Finally, we hypothesized
that the insulin-mediated suppression of plasma FFA and glycerol concentrations dur-
ing clamp conditions would accurately predict adipose tissue insulin sensitivity, and we
now demonstrate that these measurement methods accurately quantify insulin sensitiv-
ity of adipose tissue lipolysis for the first time in humans.
The rate of lipolysis may be dependent on gender and adiposity [197]. Therefore, to assess whether variation in these factors may have contributed to imprecise estimation of adipose tissue insulin sensitivity, we included these factors into the multivariate analyses. These factors did not affect the estimation of adipose tissue insulin sensitivity from 6 of the 7 tested indices, suggesting that the same indices (that is, with the same degrees of inaccuracy) can be used in adults of both sexes and across a wide range of overweight and obesity. When adipose tissue insulin sensitivity is estimated from the insulin-mediated suppression of FFA, however, values obtained in men and women may be slightly different, and the coefficients presented in Supplemental Table S2 can be used to take this into account.

A limitation of this study is that we did not include lean control subjects and, therefore, may not have captured the full biological spectrum of adipose tissue insulin sensitivity. This may limit the generalizability of our results. Although sensitivity analyses did not reveal meaningful differences in our results when analyses were repeated excluding the 4 subjects with pre-existing or de novo type 2 diabetes, we acknowledge that further studies are needed to validate the use of adipose tissue insulin resistance indices in normal-weight subjects with high adipose tissue insulin sensitivity and in patients with severely impaired adipose tissue insulin sensitivity or diabetes.

In conclusion, we demonstrate that several simplified measurement methods (indices) can accurately estimate adipose tissue insulin sensitivity. We validated the use of some of these indices in a large overweight or obese patient population. On the one hand, insulin-mediated suppressions of plasma glycerol or FFA concentrations, determined during hyperinsulinemic-euglycemic clamp experiments, very accurately reflect adipose tissue insulin sensitivity, without the need for expensive fatty acid or glycerol tracers. Fasting indices, including the Adipo-IR and insulin x glycerol, on the other hand, have lower precision, but their simplicity makes them more suitable for large-scale epidemiology as well as clinical settings. Validation of these methods is an important step towards the widespread quantification of adipose tissue function in research and/or clinical settings.

Supplemental information to this chapter is available online (http://www.nature.com/ijo).