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Modification and Validation of the Triglyceride-to–HDL Cholesterol Ratio as a Surrogate of Insulin Sensitivity in White Juveniles and Adults without Diabetes Mellitus: The Single Point Insulin Sensitivity Estimator (SPISE)

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BACKGROUND: The triglyceride-to–HDL cholesterol (TG/HDL-C) ratio was introduced as a tool to estimate insulin resistance, because circulating lipid measurements are available in routine settings. Insulin, C-peptide, and free fatty acids are components of other insulin-sensitivity indices but their measurement is expensive. Easier and more affordable tools are of interest for both pediatric and adult patients.

METHODS: Study participants from the Relationship Between Insulin Sensitivity and Cardiovascular Disease [43.9 (8.3) years, n = 1260] as well as the Beta-Cell Function in Juvenile Diabetes and Obesity study cohorts [15 (1.9) years, n = 29] underwent oral-glucose-tolerance tests and euglycemic clamp tests for estimation of whole-body insulin sensitivity and calculation of insulin sensitivity indices. To refine the TG/HDL ratio, mathematical modeling was applied including body mass index (BMI), fasting TG, and HDL cholesterol and compared to the clamp-derived M-value as an estimate of insulin sensitivity. Each modeling result was scored by identifying insulin resistance and correlation coefficient. The Single Point Insulin Sensitivity Estimator (SPISE) was compared to traditional insulin sensitivity indices using area under the ROC curve (aROC) analysis and χ^2 test.

RESULTS: The novel formula for SPISE was computed as follows: SPISE = $600 \times \text{HDL-C}^{0.185}/(\text{TG}^{0.2} \times \text{BMI}^{1.338})$, with fasting HDL-C (mg/dL), fasting TG concentrations (mg/dL), and BMI (kg/m^2). A cutoff value of 6.61 corresponds to an M-value smaller than 4.7 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (aROC, M:0.797). SPISE showed a significantly better aROC than the TG/HDL-C ratio. SPISE aROC was comparable to the Matsuda ISI (insulin sensitivity index) and equal to the QUICKI (quantitative insulin sensitivity check index) and HOMA-IR (homeostasis model assessment–insulin resistance) when calculated with M-values.

CONCLUSIONS: The SPISE seems well suited to surrogate whole-body insulin sensitivity from inexpensive fasting single-point blood draw and BMI in white adolescents and adults.

For almost 4 decades, the euglycemic hyperinsulinemic clamp test has been the gold standard for measurement of insulin sensitivity in humans *(1)*. The major interest in insulin resistance results from the fact that it is regarded as the common denominator of the (cardio-) metabolic syndrome *(2)*. However, application of the hyperinsu-

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linemic clamp test is complicated, labor-intensive, uncomfortable for the patient, and expensive. Thus, indices have been developed as surrogate measures of insulin sensitivity. Either an intravenous glucose tolerance test [for example the minimal model S_I (insulin sensitivity index) or CSI (calculated sensitivity index)] *(3, 4)* or an oral glucose tolerance test (OGTT)[14] [for example, for the Matsuda composite insulin sensitivity index (ISI)] *(5–6)* is required. But these tests are still relatively labor-intensive and expensive. Simpler approaches require a single blood draw at fasting [for example for homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI), and triglyceride-to–HDL-cholesterol (TG/HDL-C) ratio] *(7–9)*. Of note, based on HDL-C, total cholesterol, insulin, and free fatty acids, another lipid parameter–based index has been derived *(10)*. Recently, Shalaurova et al. introduced the lipoprotein resistance index, based on nuclear magnetic resonance (NMR)-derived lipoprotein information, to identify individuals with insulin resistance *(11)*.

The TG/HDL-C ratio was introduced about 2 decades ago, as a promising tool for estimating insulin resistance *(9, 12–13)*. The advantage of this ratio is the universal availability of measurement of circulating lipids in preclinical settings, such as in general practice. Thus, it is an affordable tool to estimate insulin resistance and does not include insulin. Nevertheless, the TG/HDL-C ratio shows disadvantages, such as a large variability in the proposed cutoff point of the ratio depending on population characteristics *(12)*.

We aimed to refine the TG/HDL-C ratio, using easily available data from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study cohort, a large cohort of healthy adult individuals, with the formula to predict the clamp measure of insulin resistance—the M-value.

This new TG/HDL-C ratio formula [Single Point Insulin Sensitivity Estimator (SPISE)], was then validated in both adults (RISC cohort) and adolescents enrolled in the European Union (EU) funded Beta-Cell Function in Juvenile Diabetes and Obesity (Beta-JUDO) project.

[14] Nonstandard abbreviations: OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; HOMA-IR, homeostasis model assessment–insulin resistance; QUICKI, quantitative insulin sensitivity check index; TG, triglyceride; HDL-C, HDL cholesterol; NMR, nuclear magnetic resonance; RISC, Relationship between Insulin Sensitivity and Cardiovascular Disease; SPISE, Single Point Insulin Sensitivity Estimator; EU, European Union; Beta-JUDO, Beta-Cell Function in Juvenile Diabetes and Obesity; BMI, body mass index; aROC, area under the ROC curve; M/I, M value over insulin.

Materials and Methods

STUDY POPULATIONS

RISC study population. From 2002 to 2004, 1300 adults between 30 and 60 years old were recruited from 19 centers in 14 European countries. They were all prescreened for confirmation of general good health and absence of drug intake affecting insulin sensitivity, blood pressure, circulating lipids, or glucose. Exclusion criteria were increased systolic or diastolic blood pressure ($\geq$140 or $\geq$90 mmHg) after 3 measures: hyperlipidemia (fasting circulating total cholesterol $\geq$300 mg/dL or 3.4 mmol/L and/or fasting TGs $\geq$400 mg/dL or 4.5 mmol/L), diabetes mellitus, or other chronic diseases, such as overt cardiovascular disease, >40% carotid stenosis *(14)*.

Beta-JUDO study population. The study population for index validation in adolescents is part of the Beta-JUDO study cohort (FP7-HEALTH-2011-two-stage, project number: 279153; www.betajudo.org) for an investigation taking place in 6 European countries; clinical studies for this report were carried out in Uppsala (Sweden) and Salzburg (Austria). The ages of the 30 juvenile participants were between 10 and 18 years and categorized according to the WHO criteria for overweight and obesity, being overweight with a body mass index (BMI) on or above the 90th percentile for age and sex or obese with a BMI on or above the 97th (obese) or on or above the 99th percentile (morbidly obese) for age and sex. They were healthy based on a physical examination and detailed patient history, aside from obesity and associated metabolic comorbidities. Exclusion criteria were lack of consent or any psychiatric disorder, which would hinder an informed consent and compliance to take part in the investigation.

The adolescent and the adult cohorts underwent the same diagnostics, including detailed physical examination and measures of blood pressure and anthropometry (weight, height, bioimpedance). Furthermore, all participants took part in 75-g OGTTs as well as genetic evaluations, and lifestyle questionnaires were used to detect physical activity, eating behavior, and personal and family history.

Taking into account that puberty is linked to transient insulin resistance, which decreases with the completion of pubertal development, this study included only juvenile subjects in postpubertal stages (Tanner stages 4 and 5).

Local ethics committee approval was achieved by all recruitment centers of the Beta-JUDO as well as RISC studies, and all participants gave informed written consent to the protocol. For the adolescents, written informed consent was obtained from at least one parent.

ANALYTICAL PROCEDURES

In both study cohorts, plasma HDL-C, LDL cholesterol, and triglycerides were analyzed using an enzymatic photometric test (Roche Method for Modular System; Roche Diagnostics). Insulin was analyzed by a specific time-resolved fluoroimmunoassay [AutoDELFIA (dissociation-enhanced lanthanide fluorescent immunoassay) insulin kit; Wallac Oy] in the RISC study, while single-plex ELISA kits were used (Mercodia AB®) in the Beta-JUDO cohort. All parameters were analyzed centrally.

HYPERINSULINEMIC CLAMP TEST

Euglycemic hyperinsulinemic clamp tests were applied between both studies and used to determine insulin sensitivity within an interval of maximally 3 to 4 weeks after the OGTT. After an overnight fast for at least 10 h, participants were admitted to each investigational center. Two catheters were inserted into an antecubital vein of the left and right arm for venous blood sampling and infusions, respectively. Baseline measurements of fasting circulating glucose were taken at least 3 times before clamp start. The euglycemic clamp glucose target was determined from the mean value of 3 fasting plasma glucose measurements. The glucose clamp target was set to 80 mg/dL (4.44 mmol/L) in case of a value above 80 mg/dL, and in case of a value above 100 mg/dL (5.55 mmol/L) the clamp goal was 100 mg/dL *(15–16)*. The Beta-JUDO clamp protocol was adapted to the RISC study.

Clamp tests were performed for 120 min, with primed-continuous regular insulin infusion [40 mU insulin $\cdot$ min^{-1} $\cdot$ (m^2 total body surface area)$^{-1}$ *(17)*]. Blood samples for the determination of serum insulin and C-peptide were drawn at 0 and 120 min.

CALCULATIONS AND MODELING

The M-value: 100–120 min, mg $\cdot$ kg^{-1} $\cdot$ min^{-1}; the Matsuda ISI: $10\,000/[(G_F \times I_F) \times (G_{\text{mean OGTT concentration}} \times I_{\text{mean OGTT concentration}})]^{0.5}$ *(6)*; and the HOMA-IR $(22.5/(I_F \times G_F)$ *(10)* were calculated and then compared to the novel index, where G_F was fasting glucose concentration, G_{mean} the mean glucose concentration during the OGTT, HOMA the homeostasis model assessment, I_F the fasting insulin concentration, and I_{mean} the mean insulin concentration during the OGTT.

In fact, the primary aim of this study was to generate an index for identification of as many insulin-resistant people as possible, by using only 1 blood draw. We had access to the data of 1260 nondiabetic adults of the RISC study, and to the data of 29 adolescents of the Beta-JUDO study. The index was first refined using computer-assisted mathematical modeling of data from a large, nondiabetic adult cohort that was then validated in obese adolescents.

To discriminate people who were insulin resistant from those who were insulin sensitive, we used the M-value cutoff of 4.7 mg glucose mg $\cdot$ kg^{-1} $\cdot$ min^{-1} *(17–19)*. To rule out any differences in insulin administration as well as degradation, we additionally used the M-value over insulin (M/I) with a cutoff of 8.378 as a comparison to the simple M-value. M/I was calculated as glucose disposal rate divided by the mean plasma insulin concentration during the last 20 min of the 2-h euglycemic insulin clamp [M/I (100–120 min) = (mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) per μU/mL $\times$ 100].

The source codes of our software for the development of the new index are given in the Data Supplement, Appendix 1, that accompanies the online version of this article at http://www.clinchem.org/content/vol62/issue9. In brief, in the first model, we included only TG and HDL, because they both have been initially given in the triglyceride-to-HDL-cholesterol ratio formula. In general, each modeling result was scored by correlation coefficient with M and identification of insulin resistance: The relevance of each outcome was screened by both relationship quality with M and sensitivity of insulin resistance identification. Those 2 criteria were combined to yield a score, so that the best suited outcome could be rapidly identified. So, we began to apply mathematical modeling for the TG/HDL-C ratio, using all meaningful exponents between -5 and 5 in the steps between 0.1 and 0.001 for HDL and TG. Despite moderate improvement in the correlation coefficient with M ($r = 0.328$, $P < 0.001$) when modifying the formula to $\text{HDL-C}^{0.8}/\text{TG}^{0.3}$, we were still dissatisfied and considered other easily available factors from clinical practice to be included. However, from widely used parameters in clinical practice and routine labs, only body weight and BMI were sufficiently well associated with M. Thus, we proceeded to include BMI in the formula and performed 6 further modeling runs for best definition of each exponent value for TG, HDL, and BMI. This further improved not only the correlation coefficient, but also the specificity for insulin resistance identification. Taken together, after 9 prolonged analyses, inclusion of BMI following triglycerides and HDL-C eventually yielded a markedly improved estimation of insulin sensitivity, as measured by the M-value.

Statistical analysis used SigmaPlot, IBM SPSS 2,1 and Matlab. We used an ROC curve to display the tradeoff between true positives and false positives across incremental choices of discriminating cutoff points for glucose disposal rate. The area under the ROC curve (aROC) was analyzed for all insulin sensitivity indices (SPISE, HOMA-IR, Matsuda ISI, and TG/HDL-C ratio) using the indicated M-value cutoff of 4.7 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} *(18)* or the indicated M/I-value cutoff of 8.378. Cutoff values for the insulin sensitivity indices were obtained using the maximum of the Youden-

Table 1. Clinical and biochemical characteristics of the study population.[a]

	Adult group (n = 1260)	Juvenile group (n = 29)
Age, years	43.9 (8.3)	15.0 (1.9)
Sex, % of females	55	33
BMI, kg/m^2	25.5 (4)	38.6 (10)
BMI-SDS[b]		3.3 (0.6)
TG, mg/dL	96.3 (63.1)	94.9 (35.4)
HDL-C, mg/dL	55.1 (14.7)	46.1 (13.5)

[a] Data are expressed as mean (SD) or percent of participants.
[b] BMI-SDS, BMI SD score, used in children for BMI description representing increases or decreases around the 50th percentile adjusted for age and sex.

Index [sensitivity − (1 − specificity) *(20)*]. Statistical differences in the ROC curves between insulin sensitivity indices were analyzed.

Results

ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS IN BOTH GROUPS

Adult study participants showed a mean age of 43.9 (8.3) years (55% female), whereas juveniles had a mean age of 15.0 (1.9) years (33% female). As shown in Table 1, the adult group was on average overweight, whereas the juvenile group was obese. Whereas circulating triglycerides concentrations were comparable between both groups, HDL-C concentrations were significantly lower in the juvenile group. The juvenile obese group presented with higher HOMA-IR and lower M-values (Table 2) and M/I values (data not shown), indicating a higher degree of insulin resistance in this cohort of juveniles than in the adults studied.

Table 2. Measures and estimates of insulin sensitivity in the study population derived from fasting as well as dynamic states.[a]

	Adult group (n = 1260)	Juvenile group (n = 29)
M-value, mg · kg^{-1} · min^{-1}	7.2 (3)	4.4 (1.4)
SPISE index	7.2 (2)	4.1 (1.3)
HOMA-IR	1.2 (0.7)	4.5 (2.2)
Matsuda ISI[b]	9.6 (5.5)	2.5 (1.1)
TG/HDL-C ratio	2.0 (2.3)	2.2 (0.4)

[a] Data are expressed as mean (SD).
[b] For the mean (SD) of the comparative Matsuda ISI 1200 study participants were evaluated.

Table 3. Pearson correlations of M-value to other measures of insulin sensitivity in adult and juvenile study participants.[a]

	Adult group (n = 1260) (n = 1200 for Matsuda ISI)	Juvenile group (n = 29)
	M-value, mg · kg^{-1} · min^{-1}	
SPISE index	0.474***	0.561**
HOMA-IR	−0.410***	−0.391*
Matsuda ISI	0.496***	0.319[§]
TG/HDL-C ratio	−0.237***	−0.184

Data are expressed as *r* values according to Pearson correlation calculation.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, [§]$P = 0.08$.

SPISE FORMULATION

The novel formula for SPISE was computed as follows:

$$\text{SPISE} = 600 \times \text{HDL-C}^{0.185}/(\text{TG}^{0.2} \times \text{BMI}^{1.338}), \quad (1)$$

with fasting HDL-C (mg/dL), fasting triglyceride concentrations (triglycerides; mg/dL), and BMI (kg/m^2). Although DeFronzo et al. suggested both the M-value and the M/I ratio as measures of insulin sensitivity *(1)*, we focused modeling on M-values in the Beta-JUDO cohort in this analysis.

CORRELATIONS AND PREDICTIVE VALUE OF THE SPISE INDEX

Table 3 shows the Pearson correlation coefficients for the correlation between insulin sensitivity indices and M-value. The SPISE index showed moderate to strong positive correlations with the M-value in both adults and juveniles and in each study cohort separately.

CORRESPONDING CUTOFF VALUES OF INSULIN SENSITIVITY INDICES

All participants were grouped for ROC analysis. Table 4 shows the cutoff values of the established insulin sensitivity indices compared to an M-value cutoff of 4.7 mg · kg^{-1} · min^{-1} and to a corresponding M/I-ratio cutoff of 8.387, respectively. For the SPISE index, a cutoff value of 6.61 corresponds to an M-value of 4.7 mg · kg^{-1} · min^{-1}; using M/I, the SPISE index cutoff value is 6.87, presenting with a better accuracy than when using the M-value (M/I, 0.828; M, 0.797). The other indices (Matsuda ISI, QUICKI, TG/HDL-C ratio, HOMA-IR) also show a worse accuracy using M-value compared to M/I (see Table 4). For M-value as well as M/I, the Matsuda ISI presents the best accuracy as given by aROC compared to all other indices. SPISE index, QUICKI, and HOMA-IR present with similar aROCs in both calculations. TG/HDL-C ratio shows the worst

Table 4. ROC analysis for M-value and M/I.[a]

	Insulin resistance cutoff level		Sensitivity, %		Specificity, %		aROC	
	M	M/I	M	M/I	M	M/I	M	M/I
SPISE index	6.61	6.87	66	64	80	84	0.797	0.828
Matsuda ISI	6.00	6.99	82	78	68	78	0.823	0.860
QUICKI	0.38	0.37	69	82	72	65	0.776	0.813
TG/HDL-C ratio	2.05	1.47	59	78	74	57	0.713	0.722
HOMA-IR	1.22	1.19	70	74	73	75	0.785	0.825

[a] Data are presented as cutoff values, sensitivity, specificity, and aROC. Corresponding M (4.7 mg · kg^{-1} · min^{-1}) and M/I (8.387) values were calculated for Youden index in the whole group.

accuracy compared to all other indices, when using M-value or M/I.

COMPARISON OF DIFFERENT INSULIN SENSITIVITY INDICES

With a χ^2 test, we compared aROC of the different indices using an M-value cutoff of 4.7 mg · kg^{-1} · min^{-1} as well as a corresponding M/I-ratio cutoff of 8.387. Data are depicted in Table 5. Using M-value as well as M/I ratio, SPISE index showed a significantly better aROC than the TG/HDL-C ratio [SPISE index, aROC 0.797 (M)/0.828 (M/I); TG/HDL-C ratio, aROC 0.713 (M)/0.722 (M/I); $P < 0.0001$]. Additionally, the Matsuda ISI showed significantly better accuracy than QUICKI, HOMA-IR, and TG/HDL-C ratio independent of using M or M/I ratio ($P < 0.0001$). Comparing the SPISE index and Matsuda ISI, the SPISE index aROC was comparable to the Matsuda ISI and equal to QUICKI and HOMA-IR when calculated with the M-value (SPISE index aROC, 0.797; Matsuda ISI aROC, 0.823; QUICKI aROC, 0.776; HOMA-IR aROC, 0.785; SPISE index and Matsudas ISI: $P = 0.069$). When using M/I ratio, Matsuda ISI showed better accuracy than the other indices.

Fig. 1 shows the ROC curves for all indices: the sensitivity and specificity of the SPISE index were comparable to those for the Matsuda ISI, the best among established indices. SPISE index showed a significantly better aROC than the TG/HDL-C ratio in both groups.

Discussion

The present study shows that insulin sensitivity can be estimated using the SPISE index, a new, simple index, in both adults and obese adolescents. Using an M-value derived from the euglycemic–hyperinsulinemic glucose clamp test, the accepted gold standard of insulin sensitivity, we provide evidence for the usefulness of an index integrating triglycerides, HDL-C, and body mass index. As suggested by Bergman et al. *(19)*, we used a conservative definition of insulin resistance as an M-value of <4.7 mg · kg^{-1} · min^{-1}. Given the demanding nature and the expense and time involved in performing euglycemic–hyperinsulinemic clamp tests, several indices to evaluate insulin sensitivity have been developed and validated in adult and pediatric cohorts. All of these indices have limitations (such as lack of reproducibility, methodical discrepancies between laboratories, time-consuming methods, complexity, and invasiveness), and most importantly they depict either fasting (e.g., HOMA-IR or QUICKI)

Table 5. χ^2 test and comparison of different indices using M value and M/I.[a]

		Difference aROC		Chi square		*P* value	
		M	M/I	M	M/I	M	M/I
SPISE	Matsuda's ISI	−0.026	−0.033	3.315	7.459	0.069	0.0063
SPISE	QUICKI	0.021	0.015	1.942	1.248	0.163	0.264
SPISE	TG/HDL ratio	0.084	0.106	38.744	73.450	< 0.0001	< 0.0001
SPISE	HOMA-IR	0.012	0.003	0.766	0.061	0.382	0.804

[a] χ^2 calculated using a significance level of $P < 0.0001$. Corresponding M (4.7 mg · kg^{-1} · min^{-1}) and M/I (8.387) values were calculated for Youden index in the whole group.

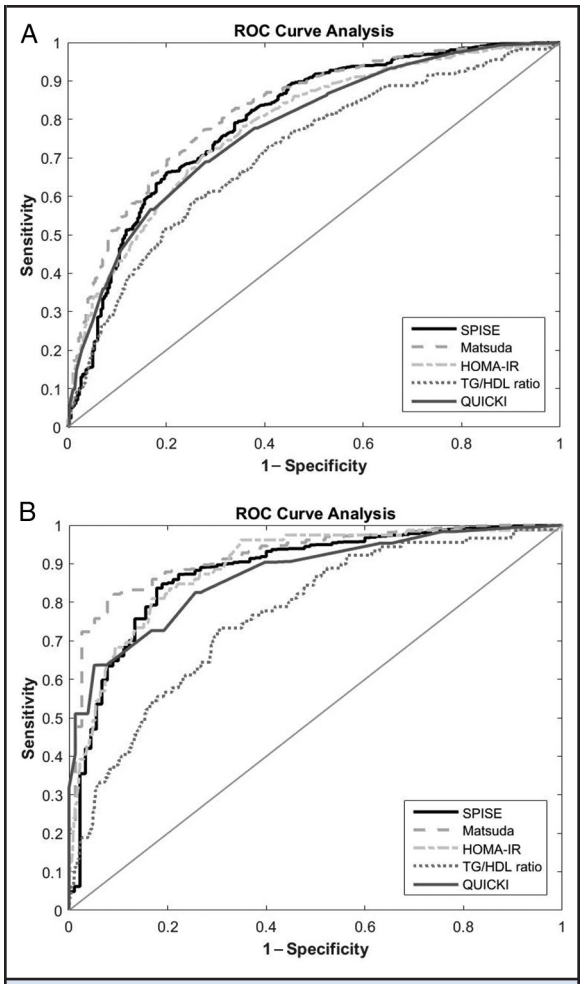

Fig. 1. (A), Summary of ROC curve analyses for SPISE index and other insulin sensitivity indices for M = 4.7 mg · kg^{-1} · min^{-1}. (B), Summary of ROC curve analyses for SPISE index and other insulin sensitivity indices for M/I = 8.387.

or dynamic states (e.g., Matsuda ISI) of insulin action separately *(3, 7–8, 21–25)*.

In addition to these traditional measures of insulin sensitivity, the TG/HDL-C ratio is characterized by the availability of measures of circulating lipids, in particular in preclinical settings *(9, 12–13, 26–29)*. Pathophysiologically, alterations in lipid and lipoprotein metabolism are among the earliest manifestations of insulin resistance *(30)*. NMR spectroscopy studies in individuals with insulin resistance have shown higher levels of VLDL and LDL particles than those found in insulin-sensitive controls *(31)*. This is due to an increase in VLDL synthesis, which has been demonstrated to be related to an increased availability of free fatty acids *(32)*, hyperglycemia *(33)*, and reduced VLDL clearance from the circulation *(33)*. Further, HDL particle levels are lower, and VLDL larger and LDL as well as HDL smaller in particle size in insulin-resistant *(34)* and prediabetic states *(35)*.

Therefore, the TG/HDL-C ratio uniquely depicts the complex associations of lipoprotein metabolism and the role of proatherogenic factors in the obesity-related cascade *(36)*. The TG/HDL-C ratio had already been presented as a promising tool for estimation of insulin resistance 20 years ago *(9, 12–13)*. However, there are large differences in the predictive power of the ratio according to ethnicity. Thus, when studying African American and other nonwhite populations, the relationship of the ratio to cardio-metabolic parameters could not be confirmed *(12, 28, 37)*. This may be due to the fact that African Americans show higher insulin levels due to a compensatory reduction in hepatic insulin extraction reducing hepatic utilization of lipids and consequently reduced circulating TG concentrations *(38)*.

In contrast, the TG/HDL-C ratio was associated with both indirect and direct measures of insulin sensitivity in all ethnic groups in pediatric study participants *(12)*. Furthermore, the TG/HDL-C ratio has been proven to be outstanding in comparison to other indicators to screen for the metabolic syndrome and was found to be a useful marker of early functional and morphological vascular changes *(28)*and cardiac remodeling, as well as nonalcoholic fatty liver disease detection in pediatric cohorts *(39)*.

McLaughlin et al. *(29)* reported that among 258 overweight individuals, fasting insulin, triglycerides, and the TG/HDL-C ratio were the best predictors of insulin resistance, as defined by the insulin suppression test. The sensitivities of their cutoff points ranged from 57% to 67% and the specificities from 68% to 85%. These results are particularly compatible with our results in obese adolescents because they apply specifically to overweight individuals, in whom the effect of BMI itself is likely to be attenuated, permitting the emergence of other predictors, in this case triglycerides and the TG/HDL-C ratio. In an analysis assembling 2321 results (2138 nondiabetic study participants) from euglycemic clamp procedures (40 mU · min^{-1} · m^{-2}), Stern et al. *(40)* found HOMA-IR, BMI, waist circumference, and LDL cholesterol to be significant predictors of insulin resistance. Tam and associates *(18)* generated classification trees for predicting insulin resistance from euglycemic clamps performed at 120 mU · min^{-1} · m^{-2} from routinely measured fasting glucose, insulin, total cholesterol, and HDL-C, thereby extending the hyperinsulinemic–euglycemic clamp from a research tool to clinical applicability. The sensitivity and specificity results from these 2 studies were similar to those calculated for the SPISE in our analysis [SPISE aROC from M-value, 0.80; M/I, 0.83; Stern et al. *(40)*: aROC, 0.90; Tam et al. *(18)*: aROC, 0.87].

The SPISE index was designed to ameliorate the TG/HDL-C ratio, to enhance its sensitivity and specific-

ity and thus provide an index without insulin as a (costly) parameter. In keeping with this goal, the SPISE performed similarly to HOMA-IR and QUICKI, but significantly better than the TG/HDL-C ratio, which showed the lowest level of agreement with the glucose clamp–derived M-value as well as the M/I-ratio (Tables 4 and 5). In addition, only OGTT-derived Matsuda ISI tended to be superior to the SPISE index (only by trend when relating to M-value, significant for M/I). Hence, similar to the current use of established indices of insulin sensitivity, the SPISE index can be used both in routine clinical practice and clinical research settings to estimate insulin sensitivity with only fasting values of triglycerides, HDL-C, and BMI needed in white children and adolescents. For the SPISE index, a cutoff value of 6.61 corresponds to an M-value of 4.7 $mg \cdot kg^{-1} \cdot min^{-1}$, smaller values indicating insulin resistance.

LIMITATIONS

Our study examined the SPISE index by investigating nondiabetic adults [mean BMI 25.5 (0.1) kg/m^2] and obese adolescents [mean BMI 38.6 (1.8) kg/m^2]. The juvenile cohort in particular represents the patient group at risk and the detection of insulin resistance is crucial for these patients. Further, ROC analyses for SPISE index and other insulin sensitivity indices showed an unfavorable kinking when using M/I as a surrogate of insulin sensitivity (data not presented). Thus, we favor using an M-value cutoff of 4.7 $mg \cdot kg^{-1} \cdot min^{-1}$, which is already the state of the art in most studies. Further analyses and studies to establish age- and sex-specific cutoffs in different patient groups are warranted. In addition, our study predominantly recruited Europeans of white ethnicity. Given the differences of lipoprotein metabolism between white and African American populations mentioned above *(12, 37, 39)*, for the time being SPISE can be applied only to white people, with validation in other populations, in particular African Americans, needed.

Conclusion

In conclusion, we have shown that the identification of insulin resistance in white pediatric and adult individuals is feasible by a simple calculation—using only routine TG and HDL-C measurements from inexpensive single-point blood samplings in fasting state and BMI. Thus, the SPISE index can aid in the identification of this important harbinger of chronic diseases and could be incorporated into clinical trials and daily clinical practice.

Appendix

RISC Investigators

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Further information on the RISC Study and participating centers can be found online at www.egir.org.

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Further information on the Beta-JUDO Study and participating centers can be found online at www.beta-judo.org.

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References

1. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–23.
2. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes 1992;41:715–22.
3. Pacini G, Tonolo G, Sambataro M, Maioli M, Ciccarese M, Brocco E, Avogaro A, Nosadini R. Insulin sensitivity and glucose effectiveness: minimal model analysis of regular and insulin-modified FSIGT. Am J Physiol 1998;274:E592–9.
4. Tura A, Sbrignadello S, Succurro E, Groop L, Sesti G, Pacini G. An empirical index of insulin sensitivity from short IVGTT: validation against the minimal model and glucose clamp indices in patients with different clinical characteristics. Diabetologia 2010;53:144–52.
5. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–70.
6. Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Jarvinen H, Van Haeften T, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000;23:295–301.
7. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
8. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402–10.
9. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL, Buring JE. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997;96:2520–5.
10. Disse E, Bastard JP, Bonnet F, Maitrepierre C, Peyrat J, Louche-Pelissier C, Laville M. A lipid-parameter-based index for estimating insulin sensitivity and identifying insulin resistance in a healthy population. Diabetes Metab 2008;34:457–63.
11. Shalaurova I, Connelly MA, Garvey WT, Otvos JD. Lipoprotein insulin resistance index: a lipoprotein particle-derived measure of insulin resistance. Metab Syndr Relat Disord 2014;12:422–9.
12. Giannini C, Santoro N, Caprio S, Kim G, Lartaud D, Shaw M, et al. The triglyceride-to-HDL cholesterol ratio: association with insulin resistance in obese youths of different ethnic backgrounds. Diabetes Care 2011;34:1869–74.
13. Kim-Dorner SJ, Deuster PA, Zeno SA, Remaley AT, Poth M. Should triglycerides and the triglycerides to high-density lipoprotein cholesterol ratio be used as surrogates for insulin resistance? Metabolism 2010;59:299–304.
14. Hills SA, Balkau B, Coppack SW, Dekker JM, Mari A, Natali A, et al. The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and objectives. Diabetologia 2004;47:566–70.
15. Anderwald C, Pfeiler G, Nowotny P, Anderwald-Stadler M, Krebs M, Bischof MG, et al. Glucose turnover and intima media thickness of internal carotid artery in type 2 diabetes offspring. Eur J Clin Invest 2008;38:227–37.
16. Anderwald C, Brabant G, Bernroider E, Horn R, Brehm A, Waldhäusl W, Roden M. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. Diabetes 2003;52:1792–8.
17. Anderwald CH, Tura A, Gessl A, Smajis S, Bieglmayer C, Marculescu R, Luger A, Pacini G, Krebs M. Whole-body insulin sensitivity rather than body-mass-index determines fasting and post-glucose-load growth hormone concentrations. PLoS One 2014;9:115–84.
18. Tam C, Xie W, Johnson W, Cefalu W, Redman L, Ravussin E. Defining insulin resistance from hyperinsulinemic-euglycemic clamps. Diabetes Care 2012;35:1605–10.
19. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocrine Rev 1985;6:45–86.
20. Youden W. Index for rating diagnostic tests. Cancer 1950;3:32–5.
21. Abbasi F, Reaven G. Evaluation of the quantitative insulin sensitivity check index as an estimate of insulin sensitivity in humans. Metabolism 2002;51:235–7.
22. Henderson M, Rabasa-Lhoret R, Bastard J, Chiasson J, Baillargeon J, Hanley J, Lambert M. Measuring insulin sensitivity in youth: how do the different indices compare with the gold-standard method? Diabetes Metab 2011;37:72–8.
23. Uwaifo G, Parikh S, Keil M, Elberg J, Chin J, Yanovski J. Comparison of insulin sensitivity, clearance, and secretion estimates using euglycemic and hyperglycemic clamps in children. J Clin Endocrinol Metab 2002;87:2899–905.
24. Yeckel C, Weiss R, Dziura J, Taksali S, Dufour S, Burgert T, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. J Clin Endocrinol Metab 2004;89:1096–101.
25. McAuley K, Williams S, Mann J, Walker R, Lewis-Barned N, Temple L, et al. Diagnosing insulin resistance in the general population. Diabetes Care 2001;24:460–4.

26. Quijada Z, Paoli M, Zerpa Y, Camacho N, Cichetti R, Villarroel V, Arata-Bellabarba G, Lanes R. The triglyceride/HDL-cholesterol ratio as a marker of cardiovascular risk in obese children: association with traditional and emergent risk factors. Pediatric Diabetes 2008;9:464–71.

27. Gaziano J, Hennekens C, O'Donnell C, Breslow J, Buring J. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circulation 1997;96:2520–5.

28. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann Intern Med 2003;139:802–9.

29. Vega G, Barlow C, Grundy S, Leonard D, DeFina L. Triglyceride-to-high-density-lipoprotein-cholesterol-ratio is an index of heart disease mortality and of incidence of type 2 diabetes mellitus in men. J Investig Med 2014;62:345–9.

30. Laakso M, Sarlund H, Mykkanen L. Insulin resistance is associated with lipid and lipoprotein abnormalities in subjects with varying degrees of glucose tolerance. Arteriosclerosis 1990;10:223–31.

31. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes 2003;52:453–62.

32. Grundy SM. Pathogenesis of hypertriglyceridemia in man. In: Carlson LA, Olsson AG, editors. Treatment of hyperlipoproteinemia. New York: Raven; 1984. p 21–34.

33. Howard BV. Lipoprotein metabolism in diabetes mellitus. J Lipid Res 1987;28:613–28.

34. Goff DC, D'Agostino RB, Haffner SM, Otvos JD, et al. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. Metabolism 2005;54:264–70.

35. Festa A, Williams K, Hamley AJ , Otvos JD, Goff DC, Wagenknecht LE, Haffner SM. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. Circulation 2005;111:3465–72.

36. Eeg-Olofsson K, Gudbjörnsdottir S, Eliasson B, Zethelius B, Cederholm J, on behalf of the NDR. The triglyceride-to-HDL-cholesterol ratio and cardiovascular disease risk in obese patients with type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). Diabetes Res Clin Pract 2014;106:136–44.

37. Maturu A, DeWitt P, Kern P, Rasouli N. The triglyceride to high-density lipoprotein cholesterol (TG/HDL) ratio as a predictor of beta-cell function in African American women. Metabolism 2015;64:561–5.

38. Sumner A, Vega G, Genoveses D, Finley K, Berman R, Boston R. Normal triglyceride levels despite insulin resistance in African Americans: role of lipoprotein lipase. Metabolism 2005;54:902–9.

39. Pacifico L, Bonci E, Andreoli G, Romaggioli S, Di Miscio R, Lombardo C, Chiesa C. Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents. Nutr Metab Cardiovasc Dis 2014;24:737–43.

40. Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. Identification of individuals with insulin resistance using routine clinical measurements. Diabetes 2005;54:333–9.