The Clamp-Like Index

A novel and highly sensitive insulin sensitivity index to calculate hyperinsulinemic clamp glucose infusion rates from oral glucose tolerance tests in nondiabetic subjects

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OBJECTIVE — Insulin resistance, the underlying pathophysiological mechanism of the metabolic syndrome, can not only predict type 2 diabetes development but also cardiovascular disease. Thus, precise insulin resistance measurement in individuals at risk for metabolic diseases would support clinical risk stratification. However, the gold standard for measuring insulin resistance, the hyperinsulinemic clamp test, is too labor intensive to be performed in large clinical studies/settings.

RESEARCH DESIGN AND METHODS — Using plasma glucose and C-peptide concentrations from oral glucose tolerance tests (OGTTs), we developed the novel "clamp-like index" (CLIX) for insulin sensitivity calculation and compared CLIX to clamp glucose infusion rates (GIR) (100–120 min). We evaluated CLIX in 89 nondiabetic subjects (58 female and 31 male, aged 45 ± 1 years, BMI 27.5 ± 0.8 kg/m^2) who underwent frequently sampled 3-h 75-g OGTTs and 2-h hyperinsulinemic-isoglycemic clamp (40 mU/min per m^2) tests.

RESULTS — CLIX, calculated as serum creatinine (×0.85 if male)/(mean $AUC_{glucose}$ × mean $AUC_{C\text{-}peptide}$) × 6,600, was highly correlated ($r = 0.670$, $P < 10^{-12}$) with and comparable to clamp $GIRs_{100-120\ min}$. In subgroup analyses, $GIRs_{100-120\ min}$ were lower ($P < 0.005$) in type 2 diabetic offspring (6.2 ± 0.7 mg · min^{-1} · kg^{-1}) than in sex-, age-, and BMI-matched subjects without a family history of type 2 diabetes (8.6 ± 0.5 mg · min^{-1} · kg^{-1}), which was also reflected by CLIX (insulin-resistant offspring 6.4 ± 0.6 vs. those without a family history of type 2 diabetes 9.0 ± 0.5; $P < 0.002$). When compared with normal-weight subjects (GIR 8.8 ± 0.4 mg · min^{-1} · kg^{-1}; CLIX 9.0 ± 0.5), both $GIRs_{100-120\ min}$ and CLIX of obese (5.2 ± 0.9 mg · min^{-1} · kg^{-1}; 5.7 ± 0.9) and morbidly obese (2.4 ± 0.4 mg · min^{-1} · kg^{-1}; 3.3 ± 0.5) humans were lower (each $P < 0.02$).

CONCLUSIONS — CLIX, a novel index obtained from plasma OGTT glucose and C-peptide levels and serum creatinine, without inclusion of anthropometrical measures to calculate insulin sensitivity in nondiabetic humans, highly correlates with clamp GIRs and reveals even slight insulin sensitivity alterations over a broad BMI range and is as sensitive as the hyperinsulinemic clamp test.

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Insulin resistance, the underlying pathophysiological mechanism of the metabolic syndrome, is closely associated with common metabolic and inflammatory diseases, such as type 2 diabetes, obesity, nonalcoholic fatty liver disease, and cardiovascular disease (1–3). The degree of insulin resistance in the insulin-resistant offspring of parents with type 2 diabetes, but not in humans without a family history of type 2 diabetes, serves as a predictor for later onset of the disease (4–6). Overweight or obesity also results in a tremendous fall in insulin sensitivity, combined with a markedly increased risk for type 2 diabetes and other disturbances (such as hyperlipidemia or arterial hypertension) (1,7).

Insulin sensitivity can best be measured with the labor-intensive hyperinsulinemic clamp technique, which is regarded as the gold standard (8). The considerable experience and extensive equipment required to perform this method renders the clamp test rather unsuitable for larger clinical studies or settings. However, the oral glucose tolerance test (OGTT), which is essential for diagnosis of glucose intolerance and type 2 diabetes in a clinical routine, is often also used for determination of insulin sensitivity, and several approaches have been introduced to derive information on insulin sensitivity from OGTT data, such as the oral glucose insulin sensitivity index (OGSI) (9), the Stumvoll index (10), and the Matsuda-DeFronzo index (11). In addition, simpler methods to estimate insulin sensitivity, based on circulating insulin and glucose concentrations at fasting, were developed, such as homeostasis

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Abbreviations: AUC, area under the curve; CLIX, clamp-like index; GFR, glomerular filtration rate; GIR, glucose infusion rate; HOMA, homeostasis model assessment; MCR_{est}, estimated metabolic clearance rate; OGSI, oral glucose insulin sensitivity index; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin sensitivity check index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

model assessment (HOMA) (12) or quantitative insulin sensitivity check index (QUICKI) (13), although with several limitations (14,15). Especially, the rather low sensitivity and specificity of these indexes to detect insulin resistance in nondiabetic individuals hinders routine clinical use.

All of the above methods have been validated against the glucose clamp; usually, an acceptable correlation was found. However, most of those indexes yield absolute values different from typical clamp test glucose infusion rates (GIRs) when given in milligrams glucose uptake per minute per kilogram body weight, as originally described by DeFronzo et al. (8). Thus, it would be desirable for research scientists who are experienced in the clamp technique that an index obtained from circulating hormone and metabolite concentrations during a standardized OGTT would give values in the range of GIRs to better classify the insulin sensitivity degree of a study population or even single individuals.

Therefore, we exploited a novel insulin sensitivity index, the clamp-like index (CLIX). CLIX is obtained from OGTT plasma data without inclusion of anthropometrical measures in nondiabetic subjects whose results sensitively indicate insulin resistance and yield values comparable and correlated closely with clamp GIRs.

RESEARCH DESIGN AND METHODS

— All participants (supplemental Table A1 [available in an online appendix at http://dx.doi.org/10.2337/dc07-0422]) were recruited by means of local advertising and were of Caucasian origin. Subjects had been instructed to refrain from excessive physical exercise and to ingest an isocaloric carbohydrate-rich diet 3 days before baseline examination (study day 1) and the clamp test (study day 2). All participants gave informed consent to the protocol, which was approved by the institutional ethics board.

Study day 1

After an overnight fast for at least 12 h, participants underwent a complete medical history and precise clinical examination. Thereafter, a 75-g OGTT was performed for 3 h with frequent blood sampling (0, 10, 20, 30, 40, 60, 90, 120, 150, and 180 min) for instant determination of plasma glucose and subsequent analysis of plasma hormones (16). None of the participants had diabetes. Of the participants, 15% had impaired fasting plasma glucose, 12% showed glucose intolerance in the OGTT, and 19% displayed at least one of both criteria (impared glucose metabolism) (17).

Study day 2

After another overnight fast for at least 12 h, two catheters (Vasofix; Braun, Melsungen, Germany) were inserted into one antecubital vein of the left and right arm for blood sampling and infusions, respectively. The isoglycemic clamp glucose target was determined from the mean value of three fasting plasma glucose measurements. However, in case of a value <80 mg/dl, the clamp target was set to 80 mg/dl, and in case of a value >100 mg/dl, the clamp goal was 100 mg/dl. Hyperinsulinemic-isoglycemic clamps were performed for 120 min with primed continuous insulin infusion (40 mU/min per m^2 body surface) (Actrapid; Novo-Nordisk, Bagsvaerd, Denmark) (8,18,19) that increased plasma insulin concentrations in all participants to 74 ± 2 μU/ml at 120 min. Plasma insulin concentrations were comparable among all studied subgroups (see below) within the last 60 min of the clamp test (data not shown). During the final 20-min clamp period, plasma glucose, measured every 5 min, remained stable in all participants (100 min, 88 ± 1 mg/dl; 120 min, 88 ± 1 mg/dl) and were not different among any of subgroups 1–3 (see below) (data not shown).

Plasma metabolites and hormones

Plasma glucose concentrations were measured using the glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma insulin and C-peptide concentrations were analyzed by commercially available radioimmunoassays from Linco Research (St. Charles, MO). Inter- and intra-assay coefficients of variation of both assays were 5 and 8%, respectively. Serum creatinine concentrations were measured by routine lab methods (http://www.kimcl.at/) (18).

Calculations

Whole-body insulin sensitivity was calculated as the mean glucose infusion rate (milligrams glucose per minute per kilogram body weight) during the final 20 min of the clamp test ($GIR_{100-120\ min}$) (8,18,19). Indexes of insulin sensitivity from the OGTT (OGIS at 120–180 min) (9), the Stumvoll Index (10), and the Matsuda-DeFronzo Index (11) and from fasting glucose/insulin concentrations (HOMA [12] and QUICKI [13]) were determined, as described. Concentration area under the curves (AUCs) were calculated with the trapezoidal rule. Glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease formula (20).

CLIX

We performed a sensitivity analysis of glucose and hormone data from the OGTT and $GIR_{100-120\ min}$ during the hyperinsulinemic clamp test. We found that the AUCs of glucose, insulin, and C-peptide were related in an inverse manner to clamp $GIR_{100-120\ min}$ better than single OGTT measurements (supplemental Table A2). In particular, the correlation of C-peptide AUC to $GIR_{100-120\ min}$ was superior to that of insulin (supplemental Table A2). The correlation became greater when using the inverse product of C-peptide AUC and glucose AUC ($r = 0.676$, $P < 10^{-12}$). In addition, the relation between $GIR_{100-120\ min}$ and ($AUC_{glucose} \times AUC_{C\text{-}peptide}$) was directly associated ($r = 0.314$, $P < 0.003$) with serum creatinine concentrations adjusted to sex (−15% in males) and leads to an improvement in correlation ($r = 0.726$, $P < 10^{-16}$). Thus, after checking a series of competing "models" by searching for that with the closest correlation with the clamp GIRs and the lowest percent deviation (D%), we defined the necessary OGTT time points for glucose and C-peptide measurements. Thereby, we ultimately defined the insulin sensitivity index as: CLIX $SC \times f/(mAUC_{glucose} \times mAUC_{C\text{-}peptide}) \times F$, where SC is baseline serum creatinine concentration (in milligrams per deciliter), $mAUC_{glucose}$ is the AUC of plasma glucose during OGTT from 0 min to the end of the time span (in milligrams per deciliter per minute) divided by the total amount of time, and $mAUC_{C\text{-}peptide}$ is the same for plasma C-peptide. The constant f is 0.85 for males and 1.0 for females. The correction factor F converts CLIX to clamp GIRs and is found to follow the following relationship: F $6.5 \times mAUC_{C\text{-}peptide} \times mAUC_{glucose} + 1{,}160$. The value of F depends on the OGTT sampling schedule. When glucose and C-peptide were measured until 180 min, F was ~5,900; if the test ended at 120 min, F was ~6,600 for frequent sampling, and F was ~4,500 for the simplest OGIS sampling at 0, 90, and 120 min (9).

The various individual CLIX indexes, which arose from the different sampling schedules, were divided by the relative mean CLIX value calculated in the whole

population. These ratios were related to the single ratios of individual $GIRs_{100–120\ min}$ to the mean $GIRs_{100–120\ min}$. This allowed the estimation of D% as an index of the goodness of the agreement of the various CLIX indexes with the clamp. D% was also calculated for HOMA and QUICKI.

When calculating CLIX with mAUCs of glucose and C-peptide obtained at four different intervals [*1*) 0, 10, 20, 30, 40, 60, 90, 120, 150, and 180 min; *2*) 0, 10, 20, 30, 40, 60, 90, and 120 min; *3*) 0, 30, 60, 90, and 120 min; and *4*) 0, 90, and 120 min], D% was 34.4, 35.1, 35.2, and 37.4%, respectively. D% of $OGIS_{120\ min}$, $OGIS_{180\ min}$, estimated metabolic clearance rate (MCR_{est}), Matsuda-DeFronzo Index, HOMA, and QUICKI were 53.2, 53.1, 40.5, 41.8, 53.1, and 58.4%, respectively. Because D% was only 0.8% higher when using 5 instead of 10 measurement points, we chose CLIX with the schedule 0, 30, 60, 90, and 120 min as the most acceptable model. Of note, we also used this method to calculate D% of GIRs in two separate clamps in 32 identical subjects (21), resulting in D% of 23.4%. When calculating CLIX with the mAUC (taken from 0 to 180 min and 0, 30, 60, 90, and 120 min) of OGTT plasma insulin instead of that of C-peptide ("$CLIX_{insulin}$," correction factor *F* would be 32,000), we found a less tight correlation between $CLIX_{insulin}$ and $GIR_{100–120\ min}$ ($r = 0.57$, $P < 10^{-8}$), with D% of 39 and 41%, respectively.

Validation of CLIX

To validate CLIX in a separate dataset from the one in which CLIX was derived, 13 nondiabetic subjects (8 female and 5 male subjects aged 46 ± 4 years [range 23–62], BMI 30 ± 3 kg/m^2) underwent a frequently sampled OGTT, following a 40 mU/min per m^2 hyperinsulinemic clamp test (as stated above).

Multiple regression analyses

Multiple linear regression analysis, based on data of all participants using $GIR_{100–120\ min}$ as a dependent variable, was applied (supplemental Table A3). Variables correlating with $GIR_{100–120\ min}$ on a level of $P < 0.05$ were considered for the first model (one covariate per 10 participants) to find possible predictors for $GIR_{100–120\ min}$. In a second model, we included those indexes for assessment of insulin sensitivity that did not directly depend on BMI (because BMI was already included with CLIX, HOMA, $OGIS_{120\ min}$, and the Matsuda-DeFronzo Index) to find out which were suitable predictors of $GIR_{100–120\ min}$. As HOMA and QUICKI and also $OGIS_{120\ min}$ and $OGIS_{180\ min}$ were based on similar calculation methods, only HOMA and $OGIS_{120\ min}$ were used for the model to avoid collinearity among covariates. The final models were verified by backward stepwise linear multiple regression analysis.

Subgroups

For evaluation of CLIX, we devided the study participants into three subgroups for possible major anthropometrical characteristics (supplemental Table A1) according to the presence or absence of a family history of type 2 diabetes (subgroup 1); BMI with normal-weight (defined as BMI <25 kg/m^2 [participants' range 18.8–25.0]), overweight (BMI 25–30 kg/m^2 [25.1–29.7]), obese (BMI 30–40 kg/m^2 [30.8–37.0]), and morbidly obese (BMI >40 kg/m^2 [40.1–60.7]) subjects (subgroup 2); and sex (subgroup 3). Moreover, we analyzed data from all participants divided into GFR quintiles and data from participants with impaired glucose metabolism in comparison to those with normal fasting glucose concentrations and normal glucose tolerance (17).

Statistics

Comparisons between two or more groups was performed by the two-tailed Student's *t* test or ANOVA following Bonferroni post hoc test. All data are given as means ± SE, and Pearson's product moment correlation was used to calculate linear relationships between variables. The normal range was defined between the 5th and 95th percentile (22). Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SPSS (version 13 for Windows; SPSS, Chicago, IL) and/or STATISTICA (StatSoft, Tulsa, OK).

RESULTS

Baseline data

Anthropometrical data, serum creatinine concentrations (range 0.54–1.13 mg/dl), and GFR of the study participants and subgroups are presented in supplemental Table 1. The age of all participants ranged between 24 and 61 years. Non–insulin-resistant and insulin-resistant offspring (subgroup 1) were matched for sex, age, and BMI; normal-weight, overweight, obese, and morbidly obese subjects (subgroup 2) were matched for sex and age; and female and male subjects (subgroup 3) were matched for age and BMI. Female and male subjects had a comparable GFR, whereas serum creatinine concentrations were higher by 25% ($P < 10^{-19}$) in male subjects.

OGTT

In subgroup 1 (supplemental Fig. A1*A–C*), insulin-resistant offspring showed higher post–glucose load plasma concentrations of glucose (30–150 min, each $P < 0.02$), insulin (60–150 min, each $P < 0.03$), and C-peptide (60–180 min, each $P < 0.04$) than non–insulin resistant subjects. During the OGTT in subgroup 2 (supplemental Fig. A1*D–F*), morbidly obese participants showed markedly higher (each $P < 0.01$) plasma glucose (0–120 min), insulin (0–180 min), and C-peptide (0–180 min) levels than normal-weight subjects. Overweight and obese subjects displayed higher plasma glucose, insulin, and C-peptide concentrations than normal-weight subjects at most OGTT time points (each $P < 0.05$) (supplemental Fig. A1*D-F*). Within the first 2 h of the OGTT, morbidly obese participants had increased (each $P < 0.05$) plasma glucose, insulin, and C-peptide levels than overweight subjects. Whereas plasma glucose during OGTT was not different between morbidly obese and obese subjects, the morbidly obese subjects showed higher plasma insulin (0–90 min) and C-peptide (0–120 min) concentrations (each $P < 0.05$ vs. obese). Glucose, insulin, and C-peptide AUCs during the OGTT were comparable between female and male subjects (data not shown).

Clamp GIRs and CLIX

Clamp $GIRs_{100–120\ min}$ correlated with CLIX in all participants ($r = 0.670$, $P < 10^{-12}$) and in normal-weight ($r = 0.665$, $P < 10^{-5}$), overweight ($r = 0.383$, $P < 0.05$), obese ($r = 0.469$, $P < 0.05$), and morbidly obese ($r = 0.858$, $P < 0.005$) subjects (supplemental Fig. 2*A*). There were no significant differences between clamp $GIRs_{100–120\ min}$ and CLIX values in all participants or any of the subgroups (supplemental Fig. A2*B*—*E*). In subgroup 1 (supplemental Fig. A2*C*), clamp GIRs and CLIX were lower in insulin-resistant offspring by 27.6 and 28.5%, respectively, when compared with subjects without a family history of diabetes (each $P < 0.005$). However, by using $CLIX_{insulin}$, no differences were observed between subjects without a family history

of diabetes (7.8 ± 0.5) and insulin-resistant offspring (7.5 ± 1.8, $P = 0.83$).

In subgroup 2 (supplemental Fig. A2*D*), overweight subjects tended to show both reduced $GIRs_{100–120\ min}$ (−18%, $P = 0.15$) and CLIX (−19%, $P = 0.13$), when compared with normal-weight participants. $GIRs_{100–120\ min}$ and CLIX were lower by 42 and 36%, respectively, in obese (each $P < 0.02$) subjects and were decreased by 73 and 63% in morbidly obese (each $P < 0.00005$) subjects when compared with normal-weight subjects. In comparison to overweight subjects, morbidly obese subjects also showed lower $GIRs_{100–120\ min}$ (−68%) and CLIX (−54%, each $P < 0.02$). In subgroup 3 (supplemental Fig. A2*E*), female subjects did not differ from male subjects regarding GIRs or CLIX. To examine possible effects of renal function on GIRs or CLIX, we divided all participants into GFR quintiles (supplemental Fig. A2*F*). Clamp $GIRs_{100–120\ min}$ and CLIX values were not different among any of the GFR quintiles.

In nondiabetic subjects with impaired glucose metabolism (impaired fasting glucose and/or glucose intolerance, $n = 17$) (17), CLIX (5.0 ± 0.8) was similar to $GIRs_{100–120\ min}$ (5.5 ± 0.8 mg · min^{-1} · kg^{-1}) and closely correlated with GIRs ($r = 0.762$, $P < 0{,}004$). CLIX and GIRs in the participants with impaired glucose metabolism were lower (each $P < 0.01$) than those in the remaining 72 normal glucose-tolerant subjects with normal fasting glucose concentrations (CLIX, 8.1 ± 0.4; $GIRs_{100–120\ min}$, 7.8 ± 0.4 mg · min^{-1} · kg^{-1}).

Validation of CLIX

In another group of 13 humans, whose data were not used for its derivation, CLIX (5.1 ± 0.6) was comparable to their clamp GIRs (5.0 ± 0.7 mg · min^{-1} · kg^{-1}). At a close correlation between CLIX and clamp GIRs ($r = 0.850$, $P < 0.0003$), D% was 28.5% in this dataset.

Other indexes

Using indexes such as $OGIS_{120–180\ min}$, the Matsuda-DeFronzo Index, HOMA, or QUICKI, a difference in insulin sensitivity between subjects without family history of diabetes and insulin-resistant offspring (subgroup 1) was not found, whereas Stumvoll's Index was 16% lower in insulin-resistant offspring ($P < 0.02$) (supplemental Table A1). Using $OGIS_{120–180\ min}$ in subgroup 2, overweight, obese, and morbidly obese humans had lower values than normal-weight subjects ($P < 0.001$). In addition, morbidly obese showed a lower value in $OGIS_{120\ min}$, but not in $OGIS_{180\ min}$, than overweight participants ($P < 0.02$). Applying MCR_{est} from Stumvoll's Index, the four weight classes were all different from each other (each $P < 10^{-6}$), and, strikingly, 50% of the MCR_{est} of the morbidly obese subjects were <0. Overweight and morbidly obese, but not obese, showed a lower Matsuda-DeFronzo Index than normal-weight subjects (each $P < 0.02$). HOMA values were only different ($P < 0.03$) between morbidly obese and normal-weight humans. When compared with normal-weight subjects, QUICKI was lower in overweight and morbidly obese ($P < 0.03$) subjects, and in the latter QUICKI was also lower in comparison to overweight and obese humans ($P < 0.05$).

Insulin resistance threshold

For definition of normal range of insulin sensitivity, we calculated the 5th and 95th percentiles (22) in all lean (BMI <25 kg/m^2) participants without a family history of type 2 diabetes ($n = 30$). Therewith, insulin resistance could be defined to be present when $GIR_{100–120\ min}$ and CLIX were <5.0 and 4.4 mg · min^{-1} · kg^{-1}, respectively. When applying these thresholds for subgroup 1, we found 46 and 33% of the insulin-resistant offspring to be insulin resistant when using $GIR_{100–120\ min}$ and CLIX, respectively.

Multiple linear regression analysis

Predictors of $GIR_{100–120\ min}$. Waist circumference, systolic blood pressure, A1C, HDL cholesterol, plasma glucose, insulin and C-peptide at fasting, and CLIX correlated with $GIR_{100–120\ min}$ and therefore were included in model 1. The stepwise backward regression performed with the remaining variables revealed that CLIX and waist circumference were predictors of $GIR_{100–120\ min}$ (model 1; $r^2 = 0.44$) (supplemental Table A3). After removal of the predictor waist circumference, the estimate of CLIX remained almost the same as in the first model, suggesting that CLIX is an independent predictor of $GIR_{100–120\ min}$.

Comparison of different models for calculating insulin sensitivity. Only those indexes in which calculation did not directly depend on BMI were included in this model: CLIX, $OGIS_{120\ min}$, HOMA, and the Matsuda-DeFronzo Index. After stepwise backward multiple regression analysis, only CLIX remained in model 2 ($r^2 = 0.44$) (supplemental Table A3). After removal of the other variables, the estimates of CLIX remained almost the same as model 1, suggesting that CLIX is an independent predictor of $GIR_{100–120\ min}$.

CONCLUSIONS — In the present study, we describe the development and application of the novel CLIX, obtained from serum creatinine and five glucose and C-peptide measurements of a frequently sampled OGTT, for calculation of insulin-mediated whole-body glucose utilization. CLIX does not contain any anthropometrical measurement in its formula and is as sensitive as the gold standard, the euglycemic-hyperinsulinemic clamp test, for detecting insulin insensitivity in nondiabetic individuals in several insulin-resistant states (i.e., family history of type 2 diabetes or obesity). In addition, CLIX was not different from GIRs when adjusted for GFR quintiles. For validation, we compared CLIX with clamp GIRs in another group of subjects whose data were not used for its derivation and found similar results regarding the tight correlation with and relatively low D% from clamp GIRs.

Insulin resistance is present in virtually all type 2 diabetic patients (18) and is the underlying pathophysiological mechanism of the metabolic syndrome that also occurs in nondiabetic humans (2,3). Furthermore, insulin resistance is regarded as a disease entity with an inherent predictive value for cardiovascular disease (2). To better estimate not only the risk for metabolic diseases including type 2 diabetes but also cardiovascular disease, it appears necessary to establish a sensitive measure of insulin (in)sensitivity in the entire population by using everyday routine methods with more frequent blood sampling. Therefore, we developed CLIX from data obtained by frequently sampled OGTTs. We could show that the minimum requirement for an acceptable insulin sensitivity estimation would be five samplings of plasma glucose and C-peptide. Thus, the deviation of CLIX from clamp results in this dataset was only 11.8% higher than the variability between two labor-intensive clamps (21).

For the diagnosis of glucose (in) tolerance and/or diabetes, fasting plasma glucose and $OGTT_{120\ min}$ measurements are required in nonpregnant women, whereas for examination of glucose metabolism in pregnancy and exclusion of gestational diabetes, the $OGTT_{60\ min}$

plasma glucose is also measured. For calculation of CLIX, two or three more blood samplings are needed, with far more information not only on glucose (in) tolerance but also on individual whole-body insulin sensitivity, which closely relates to clamp GIRs and could even be electronically computed by routine lab analyzers (because no anthropometrical data are to be input).

The insulin sensitivity reduction by 28% in type 2 diabetic offspring (supplemental Fig. A2*C*), as evident in the euglycemic-hyperinsulinemic clamp, was also detectable by CLIX application at the same degree, whereas most of the other frequently applied estimates (supplemental Table A1) failed. Only MCR_{est} was able to reveal a 16% difference between subjects without a family history of diabetes and insulin-resistant offspring. However, the Stumvoll Index is based on BMI and not only plasma metabolites and hormones. Young, healthy, insulin-resistant, type 2 diabetic offspring showed impaired endothelial function, indicating a premature start of vascular disease (23). Moreover, the degree of insulin resistance in insulin-resistant offspring was shown to predict later type 2 diabetes development (6).

Of note, 30–40% of our type 2 diabetic offspring were in the <5% percentile of insulin sensitivity in lean subjects without a family history of type 2 diabetes; thus, they were considered to be insulin resistant. As the lifetime risk of offspring of one type 2 diabetic parent to develop type 2 diabetes is ~40% (5,24), the insulin-resistant offspring in our study have now been recommended to more frequently undergo clinical examinations to immediately diagnose and treat type 2 diabetes for best avoidance of late complications.

We also studied insulin resistance in overweight and obesity and again found a very close correlation between clamp glucose infusion rates and CLIX. Insulin-dependent whole-body glucose utilization continuously fell between normal-weight and morbid obesity, which is also completely paralleled by CLIX (supplemental Fig. A2*D*). Stumvoll's Index (MCR_{est}) and OGIS, in part, reflected the weight-dependent decrease in insulin sensitivity. However, OGIS was only ~20% lower in morbidly obese subjects when compared with normal-weight subjects (supplemental Table A1). Because it estimates plasma glucose clearance (25), OGIS cannot display the enormous (by >60%) reduction of insulin-mediated glucose utilization in morbid obesity when compared with that in lean subjects (supplemental Fig. A2*D*), which is also revealed by CLIX use. Moreover, calculation of MCR_{est} of the morbidly obese humans yielded negative values in 50% because of the implementation of BMI into its formula. Thus, the ratio of ~1:10 (supplemental Table 1) in MCR_{est} between morbidly obese and normal-weight subjects does not reflect that of GIRs during clamp (~1:4). Using MCR_{est}, overweight subjects were also markedly different from normal-weight participants, which was not seen by the euglycemic-hyperinsulinemic clamp or by CLIX. From these results, it becomes obvious that the inclusion of BMI into the formula to calculate GIRs underestimates insulin resistance in lean persons and overestimates insulin resistance in morbidly obese subjects.

We also found no sex-related differences in clamp glucose infusion rates, which again were well related to CLIX (supplemental Fig. A2*E*). Finally, we examined CLIX in nondiabetic participants with glucose intolerance and/or impaired fasting glucose concentrations and found CLIX to be similar to the clamp GIRs, showing that CLIX predicts GIRs in all nondiabetic individuals, regardless of the presence or absence of normal glucose tolerance and fasting glucose. In addition, we also validated CLIX in another group of subjects whose data were not used for its derivation. Thereby, a close correlation was also found, and the deviation from clamp GIRs (D%) was only 5% higher than the day-to-day variation between two clamp tests.

Our newly developed CLIX is based on OGTT calculations from plasma glucose and C-peptide concentrations, which is in contrast with other frequently applied indexes that depend on OGTT plasma glucose and insulin levels (26). Insulin and C-peptide are secreted on an equimolar basis from the pancreatic β-cell but largely differ in kinetics: C-peptide is predominately cleared by the kidney, whereas insulin largely undergoes hepatic extraction (27,28). The plasma half-life of C-peptide (~34 min) in humans is much longer than that of insulin (~4 min) (29,30). Because of β-cell cosecretion, repetitive measurement of circulating C-peptide indirectly allows time course determination of circulating insulin but, due to longer half-life of C-peptide, in a less sensitive manner than measurement of circulating insulin itself. C-peptide kinetics as an indirect measure of β-cell release are used for estimation of insulin secretion and hepatic insulin extraction (16).

Insulin's biological action on insulin-sensitive tissues has a much longer half-life (~30–40 min) than that in blood because it acts in compartments other than blood without continuous degradation by the liver (31,32). Given the similar amount of biological half-life of insulin and circulating half-life of C-peptide, it appears conceivable that plasma C-peptide levels are able to better reflect insulin's bioactivity in insulin-sensitive tissues, predominantly skeletal muscle. Thus, the reciprocal value of OGTT plasma AUCs of both glucose and C-peptide appears to better relate to insulin-mediated whole-body glucose uptake in nondiabetic humans.

In addition, we also calculated CLIX with plasma insulin instead of C-peptide and found a less tight correlation with GIRs and a higher deviation from clamp GIRs, without any difference between subjects without a family history of diabetes and insulin-resistant offspring. This finding also suggests that estimation of whole-body insulin-mediated glucose uptake from OGTT data becomes more sensitive when including circulating C-peptide levels instead of insulin.

Another parameter in the CLIX formula is serum creatinine, which is not only a measure for kidney function but also is related to total body water and muscle mass (33) because it mostly derives from muscle. Since muscle mass is higher in male sex, men have higher plasma creatinine levels than women at similar renal function, as also found in our study group. Thus, sex-adjusted serum creatinine for calculation of CLIX rather serves as a measure for the amount of muscle mass, which is predominantly responsible for insulin-mediated glucose uptake (34).

However, there are some limitations in the use of CLIX because it was not evaluated in patients with diabetes, renal insufficiency, or severe hepatopathy. In liver cirrhosis, hepatic insulin extraction is reduced, resulting in higher circulating insulin levels both at fasting and under postabsorptive conditions (28). Thus, in those patients, circulating C-peptide, which is predominantly cleared by the kidney (27), would most likely not reflect insulin's prolonged bioactivity. Conversely, in patients with chronic kidney

failure, circulating concentrations of C-peptide rise, due to reduced elimination (28). As CLIX calculation depends on circulating C-peptide concentrations, we tested whether renal function affects or biases CLIX calculation. We divided our study participants into quintiles according to their GFR and again found close relationships between CLIX and clamp GIRs at the broad GFR range between 56 and 134 ml/min per 1.73 m^2. Humans, especially in the first quintile with lowest GFR range (56–80 ml/min per 1.73 m^2), also displayed comparable CLIX in comparison to clamp GIRs (supplemental Fig. A2*F*).

In patients with severely impaired kidney function without hemodialysis, insulin sensitivity calculation by CLIX could yield spurious results. However, it could be expected that in chronic kidney dysfunction, increased serum creatinine, which is in the numerator of the CLIX formula, compensates for less cleared, and thus higher, circulating C-peptide concentrations (28), put in the CLIX formula's denominator. CLIX values of those patients could therefore also be well correlated with GIRs. However, further studies would also be necessary to evaluate CLIX in patients with severe chronic kidney and liver disease.

Finally, it is important to mention that CLIX can certainly not replace the hyperinsulinemic clamp test completely because the clamp gives more detailed information not only on insulin-mediated whole-body glucose uptake but also on ability of insulin to suppress endogenous glucose production and free fatty acids, reflecting tissue-specific insulin sensitivity in liver and fat (18).

In conclusion, CLIX, a novel index obtained from plasma OGTT glucose and C-peptide and serum creatinine levels without inclusion of anthropometrical measures to calculate insulin sensitivity in nondiabetic humans, highly correlates with clamp GIRs and reveals even slight insulin sensitivity alterations over a broad BMI range, which is as sensitive as the hyperinsulinemic clamp. CLIX detects clinically significant insulin resistance (e.g., in type 2 diabetic offspring); therefore, it could facilitate the direct estimation of insulin resistance in larger clinical studies and, in turn, in clinical practice.

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