Clamp-like Index: a Novel, Highly Sensitive Insulin Sensitivity Index to Calculate Hyperinsulinemic Clamp Glucose Infusion Rates from Oral Glucose Tolerance Test in Nondiabetic Humans

Christian ANDERWALD1,2 *MD&Mpharm, Marietta ANDERWALD-STADLER3,4 *MD, Miriam PROMINTZER1 MD, Gerhard PRAGER5 MD, Martina MANDL1 MD, Peter NOWOTNY1, Martin G. BISCHOF1 MD, Michael WOLZT1,2 MD, Bernhard LUDVIK1 MD, Thomas KÄSTENBAUER3,4 DSc, Giovanni PACINI5 DSc, Anton LUGER1 MD, and Michael KREBS1 MD

1, Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Austria
2, Department of Clinical Pharmacology, Medical University of Vienna, Austria
3, 3rd Medical Department of Metabolic Diseases and Nephrology, Hietzing Hospital, Vienna, Austria
4, Karl Landsteiner Institute of Metabolic Diseases and Nephrology, Hietzing Hospital, Vienna, Austria
5, Department of Surgery, Medical University of Vienna, Austria
6, Metabolic Unit, Institute of Biomedical Engineering, National Research Council (ISIB-CNR), Padova, Italy

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Corresponding author:
Christian Anderwald, M.D., Mpharm.
Division of Endocrinology and Metabolism
Department of Internal Medicine III
Medical University of Vienna
Waehringer Guertel 18-20
A-1090 Vienna, Austria
Email: christian-heinz.anderwald@meduniwien.ac.at

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* Both authors contributed to this work in equal measure.
Abstract

Objective. Insulin resistance (IR), the underlying pathophysiological mechanism of the metabolic syndrome, could predict not only type-2 diabetes (T2DM) development, but also cardiovascular disease. Thus, precise IR measurement in individuals at risk for metabolic diseases would support clinical risk stratification. However, the “gold-standard” to measure IR, 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 (100-120min) (GIR$_{100-120min}$). We evaluated CLIX in eighty-nine nondiabetic subjects (f/m=58/31, aged:45±1years, BMI=27.5±0.8kg·m$^{-2}$) who underwent frequently-sampled three-hour-(75g)-oGTTs and two-hour hyperinsulinemic-(40mU·min$^{-1}$·m$^{-2}$)-isoglycemic clamp-tests.

Results. CLIX, calculated as serum creatinine($\times$0.85 if male)/(mean AUC$_{glucose}$×mean AUC$_{C-peptide}$)×6600, highly correlated (r=0.670, p<10$^{-12}$) with and was comparable to clamp GIR$_{100-120min}$.

In subgroup analyses, GIR$_{100-120min}$ were lower (p<0.005) in T2DM-offspring (OFF) (6.2±0.7 mg·min$^{-1}$·kg$^{-1}$) than in gender-, age- and BMI-matched subjects without T2DM family history (NOFF) (8.6±0.5 mg·min$^{-1}$·kg$^{-1}$), which was also reflected by CLIX (OFF:6.4±0.6 vs. NOFF:9.0±0.5, p<0.002). When compared to normal-weight subjects (GIR:8.8±0.4 mg·min$^{-1}$·kg$^{-1}$; CLIX:9.0±0.5), both GIR$_{100-120min}$ and CLIX of obese (5.2±0.9 mg·min$^{-1}$·kg$^{-1}$; 5.7±0.9) and morbid obese (2.4±0.4 mg·min$^{-1}$·kg$^{-1}$; 3.3±0.5) humans were lower (each p<0.02).

Conclusion: 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 as sensitively as the hyperinsulinemic clamp-test.
**Introduction**

Insulin resistance, the underlying pathophysiological mechanism of the metabolic syndrome, is closely associated with common metabolic and inflammatory diseases such as T2DM, obesity, nonalcoholic fatty liver and cardiovascular disease (1-3). The degree of insulin resistance in the insulin-resistant offspring (OFF) of parents with T2DM, but not in humans without a family history of T2DM, serves as a predictor for the later onset of the disease (4-6). Overweight or obesity also results in a tremendous fall in insulin sensitivity, combined with markedly increased risk for T2DM and other disturbances, such as hyperlipidemia or arterial hypertension (1,7).

Insulin sensitivity can be best 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 T2DM in 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 OGIS (9), Stumvoll's (10) and Matsuda-DeFronzo (11) methods. In addition, simpler methods to estimate insulin sensitivity, basing on circulating insulin and glucose concentrations at fasting, were developed, such as HOMA (12) or QUICKI (13), though with several limitations (14,15). Especially, the rather low sensitivity and specificity of those indices to detect IR in nondiabetic individuals hinders routine clinical use.

All the above methods have been validated against the glucose clamp and usually an acceptable correlation was found. However, most of those indices yield absolute values different from typical clamp-test glucose infusion rates (GIRs), when given in mg glucose uptake·min⁻¹·kg⁻¹ 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 most common range of GIRs in order to better classify the insulin sensitivity degree of a study population or even single individuals.

Therefore, for this study on insulin resistance in OFF, we exploited a novel insulin sensitivity index (“clamp-like index”, CLIX) obtained from oGTT plasma data without inclusion of anthropometrical measures in nondiabetic humans whose results sensitively indicate insulin resistance and yield values comparable and closely correlating with clamp GIRs.

**Materials and Methods**

**Study participants.** All participants (See Online Appendix Table A1 available at http://care.diabetesjournals.org) were recruited by means of local advertising and were of Caucasian origin. The subjects had been instructed to refrain from excessive physical exercise and to ingest an isocaloric carbohydrate-rich diet three 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 12h, the participants underwent a complete medical history taking and a precise clinical examination. Thereafter, an oral glucose (75g)
tolerance test (oGTT) was performed for 3h with frequent blood sampling (0,10,20,30,40,60,90,120,150 and 180min) for instant determination of plasma glucose and subsequent analysis of plasma hormones (16). None of the participants had diabetes. 15% of the participants had impaired fasting plasma glucose, 12% showed glucose intolerance in the oGTT, and 19% displayed at least one of both criteria (impaired glucose metabolism) (17).

Study Day 2. After another overnight fast for at least 12h, 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 lower than 80 mg/dl, the clamp target was set to 80 mg/dl, and in case of a value higher than 100 mg/dl, the clamp goal was then 100 mg/dl. Hyperinsulinemic-isoglycemic clamps were performed for 120min, with primed-continuous (40mU·min⁻¹·m⁻² body surface) insulin (Actrapid; NovoNordisk, Bagsvaerd, Denmark) infusion (8,18,19) that increased plasma insulin concentrations in all participants to 74±2 µU/ml at 120min. Plasma insulin concentrations were comparable among all studied subgroups (see below) within the last 60min of the clamp test (data not shown). During the final 20min clamp period, plasma glucose, measured every 5min, remained stable in all participants (100min: 88±1 mg/dl; 120min: 88±1 mg/dl), and were not different among any of the subgroups (see below) 1-3 (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 (www.kimcl.at) (18).

Calculations. Whole body insulin sensitivity was calculated as the mean glucose infusion rate (mg glucose·min⁻¹·kg⁻¹ total body weight) during the final 20min of the clamp test (GIR₁₀₀-₁₂₀min) (8,18,19). Indices of insulin sensitivity from the oGTT [Oral Glucose Insulin Sensitivity Index (OGIS 120min/180min) (9), Stumvoll's (10) and Matsuda-DeFronzo Index (11)] and from fasting glucose/insulin concentrations [HOMA (12) and QUICKI (13)] were determined as described. Concentration areas under the curve (AUC) were calculated with the trapezoidal rule. Glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula (20).

CLIX. We performed a sensitivity analysis of glucose and hormone data from the oGTT and the GIR₁₀₀-₁₂₀min during the hyperinsulinemic clamp-test. We found that the AUCs of glucose, insulin and C-peptide were related in inverse manner to clamp GIR₁₀₀-₁₂₀min better than single oGTT measurements (See Online Appendix Table A2). In particular, the correlation of C-peptide AUC to GIR₁₀₀-₁₂₀min was superior to that of insulin (See Online Appendix 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 GIR₁₀₀-₁₂₀min and (AUCglucose×AUCC-peptide) was directly associated (r=0.314, p<0.003) with serum creatinine concentrations adjusted to gender (-15% in males) and lead 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 D% (see below), we defined the necessary oGTT time points for glucose and C-peptide measurements. Thereby, we ultimately defined the insulin sensitivity index as:

\[ \text{CLIX} = \frac{SC \times f \times (\text{mAUC}_{\text{glucose}} \times \text{mAUC}_{\text{C-peptide}})}{F} \]

where SC is with baseline serum creatinine concentration (in mg/dl); mAUC_{glucose} is AUC of plasma glucose during oGTT from 0 min to the end of the time span (in mg·dL\(^{-1}\)·min) divided by the total amount of time; and mAUC_{C-peptide} is the same for plasma C-peptide. Constant f is 0.85 for males and 1 for females. The correction factor F converts CLIX to clamp GIRs and was found to follow the relationship:

\[ F = 6.5 \times \text{mAUC}_{\text{C-peptide}} \times \text{mAUC}_{\text{glucose}} + 1160. \]

The value of F depends upon the oGTT sampling schedule. When glucose and C-peptide are measured until 180 min, F resulted ~5900; if the test ends at 120 min, F was ~6600 for frequent sampling, while F was ~4500 for the simplest OGIS sampling: 0, 90, 120 min (9).

The various individual CLIX indices, arisen 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\(_{100-120\text{min}}\) to the mean GIRs\(_{100-120\text{min}}\). This allowed the estimation of the percent deviation (D%) as an index of the goodness of the agreement of the various CLIX indices with the clamp. D% was also calculated for HOMA and QUICKI.

When calculating CLIX with mAUCs of glucose and C-peptide obtained at (i) 0, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min; (ii) 0, 10, 20, 30, 40, 60, 90, and 120 min; (iii) 30, 60, 90, and 120 min; and (iv) 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, MCR\(_{\text{est}}\), Matsuda-DeFronzo-Index, HOMA, and QUICKI were 53.2%, 53.1%, 40.5%, 41.8%, 53.1% and 58.4%, respectively.

Since D% would be 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 the D% of GIRs in two separate clamps in 32 identical subjects [individual data published elsewhere (21)], resulting in D% of 23.4%.

When calculating CLIX with the mAUC (taken from 0-180 min and 0, 30, 60, 90, and 120 min) of oGTT plasma insulin instead of that of C-peptide (“CLIX\(_{\text{insulin}}\)”, correction factor F would be 32 000 then), we found a less tight correlation between CLIX\(_{\text{insulin}}\) and GIR\(_{100-120\text{min}}\) (r=0.57, p<10\(^{-8}\)) with D% of 39% and 41%, respectively.

Validation of CLIX. In order to validate CLIX in a separate dataset from the one in which CLIX was derived, 13 nondiabetic subjects [f/m=8/5; aged: 46±4 years (range: 23-62 years); BMI=30±3 kg/m\(^2\)] underwent a frequently sampled oGTT following a 40 mU·min\(^{-1}\)·m\(^{-2}\) hyperinsulinemic clamp-test (as stated above).

Multiple linear regression analysis, based on the data of all participants using GIR\(_{100-120\text{min}}\) as dependent variable was applied (See Online Appendix Table A3). Variables correlating with GIR\(_{100-120\text{min}}\) on a level of p<0.05 were considered for the first model (1 covariate per 10 participants) to find possible predictors for GIR\(_{100-120\text{min}}\). In a second model, we included those indices for assessment of insulin sensitivity that did not directly depend on BMI, because BMI was already included (CLIX, HOMA, OGIS 120 min, Matsuda-DeFronzo-Index), to find out which these were suitable predictors of GIR\(_{100-120\text{min}}\). As HOMA and QUICKI as well as OGIS 120 min and OGIS 180 min, respectively, were based
on similar calculation methods, only HOMA and OGIS 120min were used for the model to avoid collinearity amongst covariates. The final models were verified by backward stepwise linear multiple regression analysis.

Subgroups. For evaluation of CLIX, we divided the study participants into three subgroups for possible major anthropometrical characteristics (See Online Appendix Table A1), according to (i) presence or absence of family history of T2DM (OFF/NOFF) (subgroup 1); (ii) BMI, with normal weight (defined as <25 kg/m²; participants’ range: 18.8-25.0 kg/m²), overweight (25-30 kg/m²; 25.1-29.7 kg/m²) obesity (30-40 kg/m²; 30.8-37.0 kg/m²) and morbidly obese subjects (BMI>40 kg/m², 40.1-60.7 kg/m²) (subgroup 2); and gender (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 than two groups was performed by the two-tailed Student’s t test or ANOVA following Bonferroni post-hoc test, respectively. All data are given as means±SE. 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 Microsoft Excel®, 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 Tab.1. Age of all participants ranged between 24 and 61 years. NOFF and OFF (subgroup 1) were matched for gender, age and BMI; normal-weight, overweight, obese and morbidly obese subjects (subgroup 2) for gender and age; and females and males (subgroup 3) for age and BMI. Females and males had comparable GFR, while serum creatinine concentrations were higher by 25% (p<10⁻¹⁵) in males.

OGTT (Fig.1). In subgroup 1 (See Online Appendix Fig. A1 A-C), OFF showed higher post-glucose-load plasma concentrations of glucose (30-150min, each p<0.02), insulin (60-150min, each p<0.03), and C-peptide (60-180min, each p<0.04) than NOFF.

During oGTT in subgroup 2 (See Online Appendix Fig.A1 D-F), the morbidly obese participants showed markedly higher (each p<0.01) plasma glucose (0-120min), insulin (0-180min) and C-peptide (0-180min) 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) (See Online Appendix Fig.A1 D-F). Within the first two hours 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 morbid obese showed higher plasma insulin (0-90min) and C-peptide (0-120min) concentrations (each p<0.05 vs. obese).

Glucose, insulin and C-peptide AUCs during oGTT were comparable between females and males (data not shown).

Clamp GIRs and CLIX (Fig.2). Clamp GIRs₁₀₀-₁₂₀min correlated with CLIX in all participants (r=0.670, p<10⁻¹²), 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 (Fig.2A).

There were no significant differences between clamp GIRs\(_{100-120min}\) and CLIX values in all participants or any of the subgroups (See Online Appendix Fig. A2 B-E).

In *subgroup 1* (See Online Appendix Fig. A2 C), clamp GIRs and CLIX were lower in OFF by 27.6% and 28.5%, respectively, when compared to NOFF (each p<0.005).

However, by using CLIX\(_{\text{insulin}}\), no differences were observed between NOFF (7.8±0.5) and OFF (7.5±1.8, p=0.83).

In *subgroup 2* (See Online Appendix Fig. A2 D), overweight subjects tended to show both reduced GIRs\(_{100-120min}\) (-18%, p=0.15) and CLIXs (-19%, p=0.13), when compared to normal weight participants. GIRs\(_{100-120min}\) and CLIX were lower by 42% and 36%, respectively, in obese (each p<0.02) and were decreased by 73% and 63% in morbidly obese (each p<0.00005) when compared to normal weight subjects. In comparison to overweight subjects, the morbid obese showed also lower GIRs\(_{100-120min}\) (-68%) and CLIX (-54%, each p<0.02).

In *subgroup 3* (See Online Appendix Fig. A2 E), females did not differ from males regarding GIRs or CLIX.

To examine possible effects of renal function on GIRs or CLIX, we divided all participants into GFR quintiles (See Online Appendix Fig. A2 F). Clamp GIRs\(_{100-120min}\) 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\(_{100-120min}\) (5.5±0.8 mg·min\(^{-1}\)·kg\(^{-1}\)) and closely correlated with GIRs (r=0.762, p<0.0004). 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\(_{100-120min}\): 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 indices** (See Online Appendix Table A1). Using indices, such as OG1S 120min/180min, *Matsuda-DeFronzo*-Index, HOMA or QUICKI, a difference in insulin sensitivity between NOFF and OFF (*subgroup 1*) was not found, whereas *Stumvoll*-s index was 16% lower in OFF (p<0.02).

Using OG1S 120min/180min 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 OG1S 120min, but not in OG1S 180min than overweight participants (p<0.02). Applying MCR\(_{\text{est}}\) from *Stumvoll*-s index, the four weight-classes were all different from each other (each p<10\(^{-6}\)), and strikingly, 50% of the MCR\(_{\text{est}}\) of the morbidly obese subjects were below zero. Overweight and morbidly obese, but not obese, showed 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 to normal weight subjects, QUICKI was lower in overweight and morbidly obese (p<0.03), in the latter of whom 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 5\(^{th}\) and 95\(^{th}\) percentiles (22) in all lean (BMI<25 kg/m\(^{2}\)) participants without family history.
of T2DM (n=30). Therewith, insulin resistance could be defined to be present when GIR$_{100-120min}$ and CLIX were below 5.0 mg·min$^{-1}$·kg$^{-1}$ and 4.4, respectively. When applying these thresholds for subgroup 1, we found 46% and 33% of the OFF to be insulin resistant, when using GIR$_{100-120min}$ and CLIX, respectively. 

**Multiple linear regression analysis (See Online Appendix Table A3).**

**Predictors of GIR$_{100-120min}$:** Waist circumference, systolic blood pressure, HbA1c, HDL-cholesterol, plasma glucose, insulin and C-peptide at fasting, and CLIX correlated with GIR$_{100-120min}$ and were therefore included in the first model. The stepwise backward regression performed with the remaining variables revealed that CLIX and waist circumference were predictors of GIR$_{100-120min}$ (See Online Appendix Table A3; Model 1; $r^2=0.44$). 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$_{100-120min}$.

**Comparison of different models for calculating insulin sensitivity.** Only those indices, whose calculation did not directly depend on BMI, were included in this model: CLIX, OGIS 120min, HOMA, and Matsuda-DeFronzo-Index. After stepwise backward multiple regression analysis, only CLIX remained in the model (See Online Appendix Table A3; Model 2; $r^2=0.44$). After removal of the other variables, the estimates of CLIX remained almost the same as in the first model, suggesting that CLIX is an independent predictor of GIR$_{100-120min}$.

**Discussion**

In the present study, we describe the development and application of the novel “Clamp-Like Index” (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 measure in its formula, and is as sensitive as the “gold standard”, the euglycemic hyperinsulinemic clamp-test, for detecting insulin insensitivity in nondiabetic humans in several insulin resistant states, i.e. family history of T2DM or obesity. In addition, CLIX was not different from GIRs when adjusted for GFR quintiles. For validation, we compared CLIX to clamp GIRs in another group of humans, whose data were not used for its derivation, and found similar results regarding the tight correlation with and relatively low percent deviation from clamp GIRs.

Insulin resistance is present in virtually all T2DM patients (18), and 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). In order to better estimate not only the risk for metabolic diseases including T2DM, but also for cardiovascular disease, it appears necessary to establish a sensitive measure of insulin (in-)sensitivity in the entire population by using every-day routine methods with more frequent blood sampling. Therefore, we developed CLIX from data obtained by frequently sampled oGT. We could show that the minimum requirement for an acceptable insulin sensitivity estimation would be five samplings of plasma glucose and C-peptide. Then, the deviation of CLIX from clamp results in this dataset was only 11.8% higher than the variability between two labour-intensive clamps (21).

For diagnosis of glucose (in-)tolerance and/or diabetes, fasting and oGTT 120min plasma glucose measurements are required in non-pregnant people, while for examination of
glucose metabolism in pregnancy and exclusion of gestational diabetes, also the oGTT 60min plasma glucose is 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 that closely relates to clamp GIRs and could even be electronically computed by routine lab analyzers, since no anthropometrical data are to be input.

The insulin sensitivity reduction by 28% in T2DM offspring (See Online Appendix Fig. A2 C), as evident in the “gold-standard”, the clamp-test, was also detectable by CLIX application at the same degree, whereas most of the other frequently-applied estimates (See Online Appendix Table A1) failed. Only MCR\text{est}, was able to reveal a 16% difference between NOFF and OFF. However, that index by Stumvoll et al. is based on BMI and not only plasma metabolites and hormones. Young healthy, insulin resistant T2DM offspring showed impaired endothelial function, indicating premature start of vascular disease (23). Moreover, the degree of insulin resistance in OFF was shown to predict later T2DM development (6).

Of note, 30-40% of our T2DM offspring were below the 5% percentile of insulin sensitivity in lean subjects without a family history of T2DM, and thus considered insulin resistant. As the lifetime risk of offspring of one T2DM parent to develop T2DM is ~40% (5,24), those insulin resistant OFF in our study have now been recommended to more frequently undergo clinical examinations, in order to immediately diagnose and treat T2DM for best avoidance of late complications.

We also studied insulin resistance in overweight/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 (See Online Appendix Fig.A2 D). Stumvoll’s index (MCR\text{est}) and OGIS in part reflected the weight dependent decrease in insulin sensitivity. However, OGIS was only ~20% lower in morbidly obese when compared to normal weight subjects (See Online Appendix Table A1). Because it estimates plasma glucose clearance (25), OGIS cannot display the enormous (by more than 60%) reduction of insulin-mediated glucose utilization in morbid obesity when compared to that in lean subjects (See Online Appendix Fig. A2 D), which is also revealed by CLIX use. Moreover, calculation of MCR\text{est} of the morbid obese humans yielded negative values in 50%, because of the implementation of BMI into its formula. Thus, the ratio of ~1:10 (Tab.1) in MCR\text{est} between morbid obese and normal-weight subjects does not reflect that of GIRs during clamp (~1:4). Using MCR\text{est}, overweight were also markedly different from normal-weight participants, which was not seen by the clamp test or 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 found no gender-related differences in clamp glucose infusion rates, which again were well related to CLIX (See Online Appendix Fig. A2 E).

Finally, we examined CLIX in the 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 people, regardless of the presence or absence of normal glucose tolerance and fasting glucose.

In addition, we also validated CLIX in another group of humans whose data were not used for its derivation. Thereby,
also a close correlation was 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, which is in contrast to other frequently applied indices that depend on oGTT plasma glucose and insulin levels (26). Insulin and C-peptide are secreted on an equimolar basis from the pancreatic beta-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 (~34min) in humans is much longer than that of insulin (~4min) (29,30). Because of beta cell co-secretion, 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 beta-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 (approximately 30-40min) than that in blood, because it acts in compartments other from 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 AUC 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 higher deviation from clamp GIRs, without any difference between NOFF and OFF. 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 is also related to total body water and muscle mass (33), because it mostly derives from muscle. Since the muscle mass is higher in males, men have higher plasma creatinine levels than women at similar renal function, as also found in our study group. Thus, gender-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. On the other hand, 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⁻¹·1.73m⁻². Especially humans in the first quintile with lowest GFR range (56-80 ml·min⁻¹·1.73m⁻²) also displayed comparable CLIX in
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 be also well correlated with GIRs. However, further studies would be necessary to evaluate CLIX also 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 by 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 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 as sensitively as the hyperinsulinemic clamp-test. CLIX detects clinically significant insulin resistance, e.g. in type-2 diabetic offspring, and could, therefore, facilitate the direct estimation of insulin resistance in larger clinical studies and, in turn, in clinical practice.

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Reference List


