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ORIGINAL ARTICLE

A novel insulin sensitivity index particularly suitable to measure insulin sensitivity during gestation

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Abstract

Aims Insulin resistance underlies the etiology of both type 2 diabetes and gestational diabetes. In pregnancy, insulin resistance is also associated with an unfavorable metabolic programming of the fetus, potentially contributing to a higher risk of obesity and type 2 diabetes in the offspring. To assess insulin sensitivity, several methods based on glucose and insulin levels during a 75-g oral glucose tolerance test (OGTT) exist. It is unclear how they perform during pregnancy, where physiologically altered metabolism could introduce a bias.

Methods In a cohort comprising 476 non-diabetic subjects undergoing OGTT and hyperinsulinemic-euglycemic clamp (HEC), we used cross-validation to develop an insulin sensitivity index also based on non-esterified fatty acids (NEFA) that could be more robust during pregnancy (NEFA-index). We tested commonly used OGTT-based indexes and the NEFA-index in a different cohort of 42 women during pregnancy and 1 year after delivery.

Results The Matsuda and OGIS index failed to detect lower insulin sensitivity during pregnancy as compared to the follow-up OGTT 1 year after delivery ($p > 0.09$). The new NEFA-index incorporating BMI, plasma insulin and NEFA, but not glucose, clearly indicated lower insulin sensitivity during pregnancy ($p < 0.0001$). In the non-pregnant cohort, this NEFA-index correlated well with the gold-standard HEC-based insulin sensitivity index, and outperformed other tested indexes for the prediction of HEC-measured insulin resistance.

Conclusions This insulin/NEFA-based approach is feasible, robust, and could be consistently used to estimate insulin sensitivity also during pregnancy.

Robert Wagner and Louise Fritsche have contributed equally to this work.

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Aims

In both type 2 diabetes and gestational diabetes, hyperglycemia is caused by the combination of insufficient insulin secretion and insulin resistance. Assessment of insulin sensitivity is especially important during pregnancy, because insulin resistance of the mother may invoke unfavorable metabolic programing in the fetus possibly giving rise to consequences such as obesity and type 2 diabetes in the offspring [1].

The hyperinsulinemic-euglycemic glucose clamp (HEC) is considered as the gold standard to quantify insulin sensitivity [2]. However, performing a HEC is very laborious and thus, not feasible in large study populations. Moreover, performing HEC in human pregnancies is restricted by ethical reasons. Different surrogate measures of insulin sensitivity have, therefore, been developed. Such approaches include the assessment of insulin sensitivity from the minimal model, which is based on an intravenous glucose tolerance test (IVGTT) [3], from samples obtained during oral glucose tolerance test (OGTT) and from fasting blood samples. In normal physiology, insulin resistance is well reflected by higher insulin and glucose levels during the OGTT. Therefore, most equations to estimate insulin sensitivity incorporate insulin and glucose levels. These methods are supposed to provide good estimates of insulin sensitivity in healthy individuals. However, their performance is different across various stages of prediabetes [4]. During pregnancy, a physiologic state of insulin resistance develops in order to support fetal supply with nutrients and to provide optimal conditions for fetal growth [5]. Gestational diabetes mellitus (GDM) is a consequence of insulin resistance and insufficient insulin secretion [6]. Although the diabetic state is usually resolved with delivery, the mother's postpartum risk of developing metabolic syndrome and insulin resistance remains substantially elevated [7]. Studies show that early prediction of GDM is possible by using biomarkers [8–10], but to date, the 75-g OGTT is the best validated method to assess insulin sensitivity during pregnancy and after delivery [11]. However, the assessment of insulin sensitivity could be difficult with commonly used indexes in pregnant women, since glucose levels are substantially altered during a physiologic pregnancy. Fasting glucose levels drop early in the course of a pregnancy and further decrease during the last trimester [12]. The most widely used insulin sensitivity index, the Matsuda index, utilizes the inverse of the product of fasting and mean insulin as well as glucose levels to estimate insulin sensitivity. Given substantially lower fasting glucose in pregnancy, we hypothesized that the Matsuda index would overestimate insulin sensitivity in this setting. As a substitute, we propose a novel insulin sensitivity index,

using an extended scope of variables during the standardized 75 g OGTT. Given insulin's role in the suppression of lipolysis and a potential causal contribution of fatty acids to insulin sensitivity [13–15], circulating non-esterified fatty acids (NEFA) are tightly linked to insulin sensitivity. We, therefore, hypothesized that the inclusion of NEFA levels measured during an OGTT into an index could yield a more robust estimator of insulin sensitivity. Thus, we tested glucose, insulin and NEFA levels, as well as BMI, as variables in a model selection approach in subjects who underwent both HEC and OGTT. Eventually, we verified the new index with cross-validation. We also tested this index for its power to detect the change of insulin sensitivity in women undergoing an OGTT during the 24–30th weeks of gestation and 1 year after delivery.

Methods

Subjects

Participants of the training cohort were selected from the Tuebingen Family (TUEF) study [16]. Up to now, more than 3000 participants at increased risk of type 2 diabetes, who had a family history of type 2 diabetes, obesity or previously known impaired glucose tolerance, have been recruited in the ongoing study for metabolic phenotyping. Of these participants, 492 underwent a 75-g OGTT and a HEC. Of these, 476 had complete datasets for blood glucose, insulin and NEFAs, measured at 5 points during the OGTT.

The longitudinal test cohort comprised women in the ongoing PREG study that was designed to prospectively follow women undergoing an OGTT for screening of gestational diabetes between the 24th and 30th weeks of gestation, as recommended by local guidelines. Forty-two women with full 5-point-OGTT measurements undergoing OGTTs during the pregnancy and at the first follow-up visit 1 year after delivery were included in the analysis. The first OGTT was performed at the 27th week of gestation (median, interquartile range [IQR]: 26, 28). At this time point, the median age of participants was 30 years (IQR 27, 34) and the median BMI was 26.7 kg/m^2 (IQR 23.4, 30.7). Of the subjects, 16 (38 %) already had had a prior delivery. Fourteen subjects (33 %) had GDM.

The studies were approved by the Ethics Committee of the University of Tübingen, and all participants provided a written informed consent.

OGTT

All OGTTs were performed after an overnight fast with a standardized 75-g glucose solution (Accu-Chek Dextrose,

Roche) in our research facility. Blood samples were obtained before the glucose challenge (at minute 0), and at 4 time points after the glucose challenge in 30 min intervals (at 30, 60, 90 and 120 min).

HEC

Conventional HECs were conducted in 184 subjects and Botnia-clamps [17] were conducted in 291 subjects. In the Botnia-clamp, an IVGTT was performed during the first 60 min prior to the HEC. There is no relevant difference between the two methods in the measures of insulin sensitivity [18]. The median time lag between the OGTT and the HEC was 16 days (interquartile range 8–26 days).

The participants presented at the research facility after an overnight fast. Blood sampling was performed via a venous catheter in the forearm. The arm was heated with a heating pad to obtain arterialized blood samples. An infusion catheter was placed antecubitally on the other side. The rate of intravenous glucose infusion was adjusted on plasma glucose values that were determined in 5–10 min intervals during the clamp. A primed insulin infusion at a rate of 1.0 mU kg^{-1} min^{-1} was administered over 2 h. The clamp insulin sensitivity index was calculated as the ratio of glucose infusion rate and mean plasma insulin levels during the last 30 min of the clamp.

Laboratory measurements

A bedside glucose analyzer (glucose oxidase method, Yellow Springs Instruments, Yellow Springs, OH, USA) was used to determine plasma glucose concentrations. Insulin levels were analyzed by the ADVIA Centaur XP immunoassay system, and NEFA concentrations were measured enzymatically (WAKO Chemicals, Neuss, Germany). All analyzes were performed using the ADVIA 1800 analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany).

Statistical analysis

In the linear regression models, skewed variables were log-transformed to approximate normal distribution. Model selection was performed according to the principles recommended by Hastie, Tibshirani and Friedman [19, 20]. We used estimates of test error such as the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), as well as fivefold cross-validated R^2 to select the most parsimonious model in a stepwise forward selection linear regression setting. The insulin sensitivity estimates during and after pregnancy in the PREG study were compared with the Wilcoxon signed-rank test. AUC–ROC values were compared with DeLong's test for correlated ROC curves, as implemented in the pROC package in R [21].

The statistical analyzes were performed with JMP 11.2 (SAS, Carey, NC) and R 3.2.2 [22].

Results

Change in insulin sensitivity between the pregnant and non-pregnant state

We tested whether differences in insulin sensitivity during pregnancy and 1 year after pregnancy can be detected by commonly used sensitivity indexes. A comparison of anthropometric and glycemic parameters of the investigated 42 women is shown in Table 2. Unexpectedly, insulin sensitivity estimated by the Matsuda index was not significantly different 1 year after gestation, than during pregnancy ($p = 0.09$, Fig. 2). Another widely used insulin sensitivity index, the oral glucose insulin sensitivity [OGIS] index [23], also showed no difference between insulin sensitivity during pregnancy and after pregnancy in the same women ($p = 0.2$, Fig. 2). Interestingly, the commonly used HOMA-IR index, based on fasting insulin and glucose, reached marginal significance in testing the difference between the pregnant and non-pregnant state, however, in the unexpected direction. A higher HOMA-IR in the non-pregnant would suggest higher insulin resistance (lower sensitivity) during the follow-up in contrast to pregnancy. We, therefore, endeavored to develop a novel insulin sensitivity index also using NEFA levels that might detect differences in insulin sensitivity between the pregnant and non-pregnant state.

Development of a novel insulin sensitivity index based on BMI, insulin and NEFA

We performed model selection in 476 non-diabetic subjects of the TUEF cohort with 16 initial variables comprising BMI and 5-point (0, 30, 60, 90, 120 min) measurements of glucose, insulin and NEFAs during the OGTT. The dependent variable of the model was the insulin sensitivity index (ISI) derived from the HEC (HEC-ISI). Cross-validation and calculated test error estimates were used to choose the optimal model. During the stepwise addition of model variables (see Supplemental Data), the first glucose value, $glucose_{90}$, was entered only at step 7 and did not relevantly improve the model fit. The most parsimonious linear regression model comprised BMI, $insulin_0$, $insulin_{60}$, $insulin_{120}$ and $NEFA_{120}$. This optimized model (NEFA-ISI, Fig. 1a) had a cross-validated R^2 of 0.736. The R^2 between the NEFA-ISI and the HEC-ISI in the training set was 0.741.
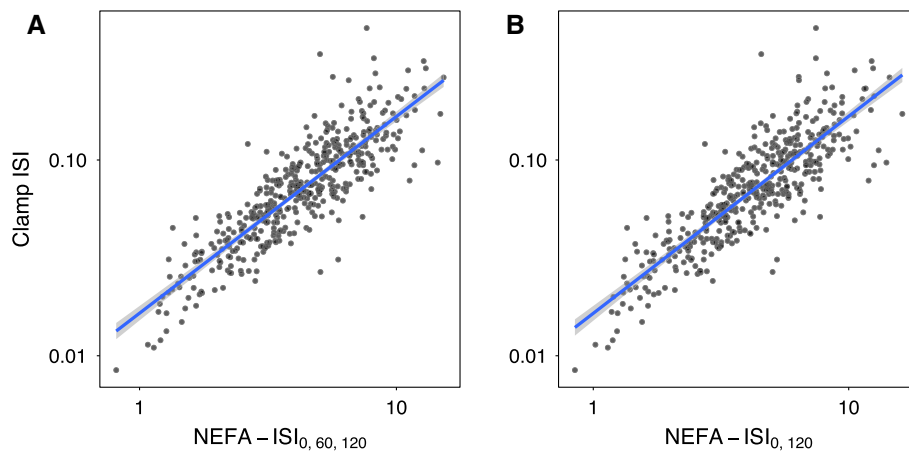
Fig. 1 Correlation of NEFA-ISI with clamp-ISI. The NEFA-ISI indexes (**a**, **b**) are calculated as. **a** $NEFA\text{-}ISI_{0,60,120} = 60 \cdot e^{3.853 - 0.9 \cdot \ln BMI - 0.205 \cdot \ln insulin_0 - 0.128 \cdot \ln insulin_{60} - 0.256 \cdot \ln insulin_{120} - 0.138 \cdot \ln NEFA_{120}}$. **b** $NEFA\text{-}ISI_{0,120} = 60 \cdot e^{3.728 - 0.96 \cdot \ln BMI - 0.252 \cdot \ln insulin_0 - 0.306 \cdot \ln insulin_{120} - 0.135 \cdot \ln NEFA_{120}}$. The clamp-ISI was calculated as the quotient of the M-value and the mean insulin level in pmol/l during the steady state of the clamp. The ln-transformed clamp-ISI regresses on the ln-transformed NEFA-ISI when insulin is given in pmol/l, NEFA in μmol/l, BMI in kg/m^2. The NEFA-ISI has been rescaled (multiplied by 60) to yield comparable numbers to the Matsuda index. Both axes are log-scaled

When testing an extended scope of variables, also including age and gender, these variables entered the model only at step 8 and 15, respectively.

Model selection was also performed on variables of restricted measurement time points. Here, we used a simplified 2-point OGTT measurement set at 0 and 120 min. The regression equations from the model selection algorithm similarly comprised BMI, insulin at two time points and $NEFA_{120}$. This estimate yielded a marginally lower correlation with the HEC-ISI (Fig. 1b) than the NEFA-ISI utilizing measurements at 0, 60, 120 min (Fig. 1a). The indexes are shown in the legend of Fig. 1 and can also be calculated with the supplied Excel table.

All of our indexes showed robust correlations with the HEC-ISI [Pearson's correlation coefficient (r) for the NEFA-ISI$_{0,60,120}$ 0.860 (CI 0.834–0.881), and the 2-point NEFA-ISI$_{0,120}$ 0.853 (CI 0.827–0.876)]. Comparing traditional insulin sensitivity indexes revealed numerically lower correlations with the HEC-ISI. The Matsuda index [24] had an r of 0.812 (CI 0.779–0.841) and the OGIS [23] an r of 0.677 (CI 0.625–0.723). The revised QUICKI, as an index utilizing fasting NEFA values, but no post-challenge variables, had an r of 0.55 (CI 0.48–0.61).

The performance of these indexes in the prediction of insulin resistance, defined as the lower quartile of HEC-ISI, was also assessed through the calculation of AUC–ROC values. This is shown in Supplementary Figure 1. A comparison of AUC–ROC of the NEFA-ISI$_{0,60,120}$ (0.94) with that of the Matsuda index (0.91) and the OGIS (0.86) showed a superiority of the NEFA-ISI$_{0,60,120}$ in both comparisons ($p = 0.004$ and $p < 0.0001$, respectively).

Furthermore, the NEFA-ISI$_{0,60,120}$ had consistently good correlations with the HEC-ISI in all stages of glucose tolerance and BMI groups (see Table 1 and Supplementary Figure 2.). Generally, a somewhat higher correlation of OGTT-based indexes in the obese (BMI ≥ 30 kg/m^2) compared to the non-obese was observed with all examined indexes.

In contrast to indexes based on insulin and glucose measurements during the OGTT (see above), the NEFA-ISI successfully detected the expectedly higher insulin sensitivity at follow-up after pregnancy ($p < 0.0001$, Fig. 2). As shown in Table 2, due to an additional body weight of 7.2 kg at the time of OGTT during pregnancy, the calculated BMI was significantly higher during the pregnancy visit. However, this difference mostly reflected the weight of the feto-placentar unit, which is metabolically not necessarily comparable to adiposity-related excess weight. Therefore, we investigated whether the good performance of the NEFA-index solely relies on the incorporation of BMI. When calculating NEFA-ISI in pregnancy using the respective post-pregnancy BMI instead of the actual BMI, we still found significantly lower NEFA-ISI in pregnancy ($p < 0.0001$).

Conclusions

It has been shown with the HEC technique that insulin action in normal pregnancy is 50–70 % lower than in non-pregnant women [25]. In the present study, however, the commonly used Matsuda index and OGIS index failed to capture the difference in insulin sensitivity between

Table 1 Correlation of insulin sensitivity indexes (ISI) with the clamp-based insulin sensitivity index in the whole cohort and in subgroups stratified on glycemic as well as BMI categories (Pearson's correlation coefficients, their 95 % confidence intervals and Spearman's rho shown in italic)

	NEFA-ISI$_{0,60,120}$	NEFA-ISI$_{0,120}$	Matsuda-ISI	OGIS
all $n = 476$	0.86	0.85	0.81 (0.78–0.84)	0.68
	(0.83–0.88)	(0.83–0.88)	*0.81*	(0.62–0.72)
	0.86	*0.85*		*0.70*
NGT $n = 362$	0.82	0.81	0.78	0.62
	(0.78–0.85)	(0.77–0.84)	(0.73–0.82)	(0.55–0.68)
	0.82	*0.80*	*0.77*	*0.65*
IFG $n = 35$	0.89	0.88	0.87	0.77
	(0.79–0.94)	(0.77–0.94)	(0.76–0.94)	(0.58–0.88)
	0.86	*0.86*	*0.82*	*0.72*
IGT $n = 47$	0.83	0.86	0.72	0.57
	(0.71–0.90)	(0.76–0.92)	(0.54–0.83)	(0.33–0.73)
	0.81	*0.82*	*0.71*	*0.61*
IFG + IGT $n = 32$	0.87	0.85	0.75	0.59
	(0.75–0.94)	(0.72–0.93)	(0.54–0.87)	(0.30–0.78)
	0.84	*0.85*	*0.68*	*0.58*
Non-obese $n = 346$	0.77	0.76	0.74	0.57
	(0.72–0.81)	(0.71–0.80)	(0.69–0.78)	(0.50–0.64)
	0.77	*0.75*	*0.74*	*0.59*
Obese $n = 130$	0.86	0.86	0.77	0.62
	(0.80–0.90)	(0.80–0.90)	(0.69–0.83)	(0.51–0.72)
	0.85	*0.85*	*0.76*	*0.63*

Fig. 2 Change in OGTT-based insulin sensitivity from pregnancy to follow-up (1 year after delivery) as estimated by the Matsuda index, OGIS, and the NEFA-index in 42 women. *Blue lines* represent increasing, *red lines* decreasing insulin sensitivity. The difference was tested with Wilcoxon's signed-rank test (color figure online)
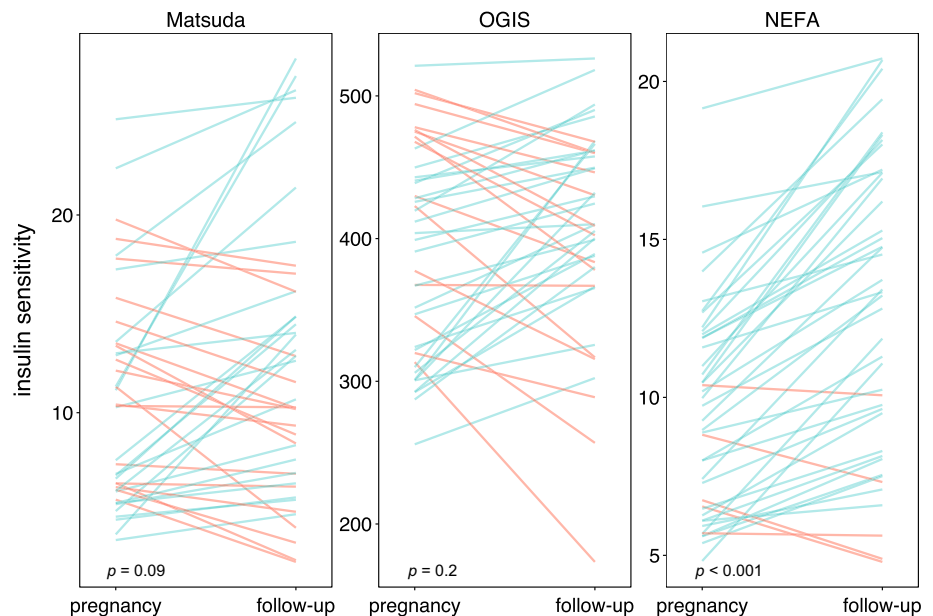
pregnant and non-pregnant women. We, therefore, sought to develop an index based on OGTT measurements, also including NEFA levels during the OGTT. This index performed at least as good as previous OGTT-derived indexes in a non-diabetic cohort, over a broad range of different phenotypes. Most importantly, the NEFA-index was able to capture the difference in insulin sensitivity between the pregnant and non-pregnant state. Of note, there is still a

considerable variability in the change in insulin sensitivity between the pregnant state and one year after pregnancy (Fig. 2) which may be determined by various hormonal and metabolic factors in pregnancy and post-pregnant status.

We propose that an improved estimation of insulin sensitivity from OGTT during pregnancy should also include NEFAs. Belfiore et al. [26] were among the first to introduce NEFAs as an explanatory variable in the assessment of

Table 2 Anthropometric and metabolic variables of 42 women during pregnancy and follow-up (1 year after delivery)

	Mean/n (%) pregnancy	Mean/n (%) follow-up	Mean difference (SD)	p value*
Age (years)	31	32	1.3 (0.46)	<0.0001
Weight (kg)	78	71	−7.2 (5.1)	<0.0001
BMI (kg/m²)	28	25	−2.5 (1.8)	<0.0001
Fasting glucose (mmol/l)	4.5	5	0.49 (0.39)	<0.0001
Post-challenge glucose 60 min	8.6	7.2	−1.5 (1.8)	<0.0001
Post-challenge glucose 120 min	6.8	5.9	−0.86 (1.4)	0.0003
Fasting insulin (pmol/l)	76	86	9.7 (49)	0.2
Post-challenge insulin 60 min	867	654	−213 (560)	0.02
Post-challenge insulin 120 min	706	548	−158 (684)	0.0007
Fasting NEFA (µmol/l)	472	661	189 (297)	0.0006
Post-challenge NEFA 60 min	264	130	−133 (520)	0.002
Post-challenge NEFA 120 min	91	66	−25 (117)	0.0001
HOMA-IR	2.2	2.8	0.56	0.03
Insulin sensitivity (Matsuda index)	11	12	1.8 (5.2)	0.09
Insulin sensitivity (OGIS index)	391	407	16 (70)	0.2
Insulin sensitivity (NEFA-index)	9.5	13	3.3 (2.7)	<0.0001

* Calculated with Wilcoxon's signed-rank test

insulin sensitivity. Fasting NEFAs are also incorporated in the revised QUICKI [27], and the adipocyte insulin resistance index proposed by Abdul-Ghani et al. [28]. During our stepwise model selection approach, glucose levels did not relevantly improve model performance. An estimation of insulin sensitivity without using glucose levels seemed to be especially suitable to assess insulin sensitivity in pregnancy, because glucose levels are altered during the gestation. Most importantly, fasting glucose is substantially decreased during pregnancy due to dilution effects secondary to increased maternal plasma volume [29] and increased glucose utilization by the growing fetus [12].

The novel NEFA-index detected the physiologic difference of insulin sensitivity in the follow-up examination of a pregnant cohort, which is to be expected [5, 30]. In contrast to that, insulin sensitivity indexes using glucose and insulin data during the OGTT did not capture the difference in insulin sensitivity between pregnant and non-pregnant women.

The data presented in the Supplementary Figures 1 A–B suggest that a lower variation of the NEFA-index has led to a superior sensitivity in pregnancy. We speculate that post-challenge NEFA levels are less affected by pregnancy-dependent factors that are not closely related to insulin sensitivity, but influence glucose levels. For example, glucose levels during the OGTT are determined by the rate of intestinal glucose absorption, the suppression of endogenous glucose production and the rate of peripheral glucose uptake in response to insulin. In contrast, post-challenge NEFA levels are a consequence of insulin action

in liver and predominantly in adipose tissue, i.e., the suppression of lipolysis.

OGTT-based indexes have not been validated in patients with diabetes. The consistently good performance of the NEFA-index in prediabetes, especially in the combination of IFG and IGT, that is considered to be close to manifest diabetes, could conceivably lead to a good model performance, even in subjects with mild forms of metabolically stable incident diabetes. These questions should be elaborated by further studies.

Disadvantages of the NEFA-index in the estimation of insulin sensitivity comprise the higher cost of NEFA-measurement and the necessity for careful sample preprocessing to avoid in vitro lipolysis and hence, falsely elevated NEFA levels [31]. However, if properly handled, NEFA can be accurately measured from stored frozen EDTA plasma samples at a later time point [32].

The NEFA-index seems to be a sensitive tool for detecting insulin resistance in pregnancy. In the scientific setting, its most important role could be a more accurate investigation of the consequences of maternal insulin resistance on fetal outcomes. Clinically, a screening for impaired insulin sensitivity during pregnancy might help identify women at risk to develop GDM later in pregnancy (beyond gestational week 28), when an OGTT is not routinely performed.

A limitation of our study is the lack of an independent replication cohort with HEC. An important caveat by using the same cohort for training and testing a model is overfitting, which could lead to an overestimation of model

performance in the training set. However, all variables used in our models have sound physiological indication and are already widely used in the estimation of insulin sensitivity. We applied a cross-validation technique which produced similar errors in the training and test sets. Restrictiveness in adding further features to the models also confines variance. To clearly demonstrate the longitudinal change in insulin sensitivity related to pregnancy and validate OGTT-based variables, a cohort with both HEC and OGTT data including NEFA measurements during and after pregnancy would have been needed. However, we were not able to perform HEC in pregnant women in our academic setting.

In summary, we propose new models for the estimation of insulin sensitivity from the OGTT that do not utilize glucose measurements and are solely based on BMI, insulin and NEFA levels. These models seem to outperform previous indexes during gestation, and provide solid performance in different prediabetes stages, as well as in obese and lean subjects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The studies were approved by the local institutional review board.

Human and animal rights disclosure All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

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