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Short title: Insulin sensitivity in minipigs.

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List of abbreviations.

ACPR$_G$  acute C-peptide response to glucose
AIR$_G$  acute insulin response to glucose
AUC  area under the curve
BW  body weight
CV%  coefficient of variation
$G_b$  basal (fasting) glucose concentration
$G_m$  mean glucose concentration
HED  high energy diet
$I_m$  mean insulin concentration
$I_b$  basal (fasting) insulin concentration
ISI$_{comp}$  insulin sensitivity index from oral glucose (Matsuda index)
ISI$_{ITT}$  insulin sensitivity index from the insulin tolerance test
ITT  insulin tolerance test
IVGTT  intravenous glucose tolerance test
$K_G$  intravenous glucose tolerance index
LED  low energy diet
M  mean glucose infusion rate during steady state in hyperinsulinemic, isoglycemic clamp
M/G/I  insulin sensitivity index from hyperinsulinemic, isoglycemic clamp
mITT  modified insulin tolerance test
OGIS  oral glucose insulin sensitivity
OGTT  oral glucose tolerance test
QUICKI  quantitative insulin sensitivity check index
$S_t$  minimal model insulin sensitivity index derived from a 2 h IVGTT
S2  new insulin sensitivity index derived from a 30 minute IVGTT
Vd  distribution volume
$\Delta$  relative difference from fasting level
Abstract

The use of animal models in diabetes research requires reliable tests for evaluation of insulin sensitivity and beta cell function. Minipigs are being increasingly used in metabolic research, and the aim of this study was to compare different tests and indices for evaluation of insulin sensitivity and beta cell function in Göttingen minipigs.

Hyperinsulinemic, isoglycemic clamp, intravenous (IVGTT) and oral (OGTT) glucose tolerance tests and modified insulin tolerance test were performed in minipigs fed either low or high energy diet. Furthermore, the reproducibility of IVGTT derived parameters was assessed.

Previously described insulin sensitivity indices (steady state glucose infusion rate/glucose concentration/insulin concentration from clamp (M/G/I); oral glucose insulin sensitivity (OGIS) and ISI\textsubscript{comp} from OGTT; S\textscript{1} from minimal model analysis of IVGTT and quantitative insulin sensitivity check index from fasting values) were calculated together with an insulin sensitivity index from the modified insulin tolerance test, ISI\textsubscript{ITT}, and a new simple index, S\textsubscript{2}, derived from the first 30 min of the IVGTT. Beta cell function was assessed from the IVGTT and the OGTT.

Reproducibility of the IVGTT derived parameters was calculated as median intraindividual CV%.

M/G/I correlated significantly only with S\textsubscript{2} (p<0.05, r=0.54). S\textsubscript{2} furthermore correlated with S\textsubscript{1} (p<0.001, r=0.81), ISI\textsubscript{ITT} (p<0.001, r=0.57) and the two indices from OGTT, ISI\textsubscript{comp}(p<0.001, r=0.78) and OGIS (p<0.05, r=0.48). No correlation was found between beta cell function indices from OGTT and IVGTT. The median CV% of the new S\textsubscript{2} index was 13.

In conclusion, the new simple index of insulin sensitivity, S\textsubscript{2}, revealed to be useful for evaluation of insulin sensitivity in pigs.
Key words: glucose tolerance, beta cell function, IVGTT, OGTT, clamp
**Introduction**

Type 2 diabetes is characterized by glucose intolerance caused by a combination of markedly reduced insulin sensitivity and insulin deficiency relative to the degree of insulin resistance (7, 20). It is therefore of great importance to reliably quantify these two parameters not only in humans but also in animal models of type 2 diabetes. Several methods to evaluate insulin sensitivity and beta cell function have been employed and validated in humans (33) and in rodents (34). These include fasting and dynamic measures obtained from oral and intravenous glucose tolerance tests as well as insulin tolerance tests (4, 5, 27-29, 33, 37). The hyperinsulinemic, iso- or euglycemic clamp is considered the “gold standard” for evaluation of insulin sensitivity (11, 33); however, this test is quite laborious.

Pigs are being increasingly used as an animal model within obesity and diabetes research; and the Göttingen minipig is one of the more extensively utilized strains (19, 22, 23, 26, 36). Various tests and indices for measuring insulin sensitivity in pigs have been exploited (12, 19, 24, 31, 38, 42) but to our knowledge these have never been validated against the hyperinsulinemic, isoglycemic clamp. This is necessary since this type of animal model is likely to be more employed in studies requiring metabolic measurements.

The aim of the present study was to compare different indices from oral and intravenous glucose tolerance tests as well as from insulin tolerance test with those obtained from the hyperinsulinemic, isoglycemic glucose clamp in Göttingen minipigs of different degree of obesity and insulin resistance. Following this analysis, a new and simple method to evaluate insulin sensitivity was derived from a short intravenous glucose tolerance test. The new index emanating from this simple test was validated and its reproducibility assessed.
Materials and methods

Animals.

Fourteen female and 11 male Göttingen minipigs 7 weeks of age at the beginning of the study were purchased from a microbiologically defined barrier unit (Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark, [www.minipigs.dk]) and used for evaluating 4 different in vivo tests for the measurement of insulin sensitivity. Other 6 approximately 12 months old male Göttingen minipigs were used for the reproducibility study. Characteristics of this pig strain have been described in detail earlier (10, 15). The pigs were allowed an acclimatization period of 3 weeks during which they were gradually introduced to their diets and trained in the experimental procedures. All animals were housed in single pens under controlled conditions and specially trained personnel cared for the animals. The study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Diet and feeding regimen.

The 25 pigs in the insulin sensitivity study were fed twice daily with a restricted amount of either low energy diet, LED (low fat, high fiber diet, 6 males and 7 females) or high energy diet, HED (high fat, high sucrose diet, 5 males and 7 females); all had free access to water. The experimental diets, purchased from Brogaarden (Gentofte, Denmark), were given in the milled form (Table 1).

The 6 pigs for the reproducibility study were fed twice daily with a mixture of 140 g minipig diet from SDS (Special Diets Services, Essex, England) and 265 g commercial swine fodder (Antonio, Slangerup, Denmark) throughout the study.
Implantation of central venous catheters.

To be able to take blood samples without stressing the animals, two central venous catheters (Cat n. C-TPNS-6.5-90-REDO, William Cook, Bjaeverskov, Denmark) were surgically implanted under general anesthesia. The animals were anesthetized IM with a mixture containing zolazepam and tiletamin (0.81 mg/kg of both zolazepam and tiletamin, Zoletil® 50 Vet, ChemVet, Denmark), ketamin (0.81 mg/kg, Ketaminol® Vet, 100 mg/mL, Intervet, Denmark), xylazin (0.84 mg/kg, Rompun® Vet, 20 mg/ml, Bayer, Denmark) and buprenorphin (0.16 mg/kg, Torbugesic®, 10 mg/mL, Scanvet, Denmark) and 0.05 mg/kg Atropin (Atropin DAK, 1 mg/mL, Nycomed, Denmark). To allow for tracheal intubation the animals were given 1 mg/kg propofol (Rapinovet, 10 mg/mL, Schering-Plough A/S, Denmark) and during the surgical procedure they were maintained on inhalation of a combination of 1.5-2.5% isoflurane (Forene®, Abbott, Denmark) and oxygen. Preoperatively the animals were given an intramuscular injection of 0.4 mg/kg carprofen (Rimadyl® Vet. 50 mg/ml, Pfizer, Denmark) and 25 mg/kg dihydrostreptomycin + 20,000 IE/kg benzylpenicillinprocain (Streptocillin® Vet., 250 mg dihydrostreptomycin + 200,000 IE benzylpenicillinprocain per mL, Boehringer Ingelheim, Denmark). Postoperatively before the end of anesthesia the animals were given an intramuscular injection of 0.015 mg/kg buprenorfine (Anorfin®, 0.3 mg/ml, GEA, Denmark), and for 3 days post operatively they were given a daily intramuscular injection of 0.4 mg/kg carprofen and 25 mg/kg dihydrostreptomycin + 20,000 IE/kg benzylpenicillinprocain to prevent post-surgical pain and infection.
In vivo tests for evaluation of insulin sensitivity and beta cell function.

The tests for evaluation of insulin sensitivity and beta cell function were performed over a period of 3 weeks, starting after 3½ months of diet feeding and in all cases after an 18 h overnight fast.

The pigs used in the reproducibility study had the IVGTT performed twice 5 days apart.

Mixed meal oral glucose tolerance test (OGTT)

The OGTT was performed in 23 animals. Immediately after three basal samples (t = -15, -5, 0 min), pigs were given a mixed meal glucose load consisting of 2 g/kg glucose (Glucose 500 g/L, SAD, Denmark) mixed with 12 g of SDS minipig diet; the meal was consumed in approximately 1 minute. Blood samples were then collected at 15, 30, 45, 60, 90, 120, 150 and 180 min.

Intravenous glucose tolerance test (IVGTT)

An intravenous glucose bolus of 0.3 g/kg (Glucose 500 g/L, SAD, Denmark) was administered in 25 pigs over 30 seconds after collection of basal samples at -20, -10, -3, -2 and 0 min. Blood samples were then collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 30, 45, 60, 90 and 120 min. In the reproducibility experiment blood samples were taken at -10, -5, 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min.

Modified insulin tolerance test (mITT).

The mITT, modified after Otis et al. (31), was performed in 22 pigs. After basal samples an intravenous bolus of 5 μg/kg somatostatin (Sigma S9129, 25 μg/ml 0.9% sterile saline) was given at -5 min. At the same time a continuous infusion of 100
mg/kg/hour somatostatin (Sigma S9129, 25 μg/ml 0.9% sterile saline) was started and continued for 35 min. An intravenous bolus of 0.5 g/kg glucose (Glucose, 500 g/L SAD) was given at 0 min and an intravenous bolus of 0.05 U/kg human insulin (100 U standard, Novo Nordisk A/S, diluted to a concentration of 0.5 U/ml in a 50 mM Na-phosphate buffer containing 0.07% polysorbate 20) was given at 15 min. Blood samples were collected at -15, -5, 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 min.

**Hyperinsulinemic, isoglycemic clamp**

Animals (n=17) were clamped on their individual fasting blood glucose level (minimum 3.5 mmol/l), which was an average of 3 fasting blood samples taken immediately before the clamp (-30, -15, -5 min). Insulin infusion (2mU/kg/min, human insulin solution prepared as described above) was initiated at 0 min and the glucose infusion (12-20 ml/hour of a 200 mg/ml solution) 5 min afterwards. The glucose infusion rate was regulated on the basis of frequent plasma glucose measurements to keep the pigs within ±0.5 mmol/l of their fasting glucose level. Blood samples of 0.8 ml each were collected in heparin coated glasses for measurement of glucose every 15-20 minutes: blood was centrifuged for 1 min and plasma glucose level immediately measured on a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA) using the glucose oxidase method. Samples for insulin measurement were taken at 15, 30, 60, 90, 120, 150, 180 and 210 min. Glucose infusion rate (M, mg/kg/min) together with mean glucose (Gm) and mean insulin (Im) concentrations during the last 30 minutes of the clamp were used to assess clamp insulin sensitivity (M/G/I).
Handling and analysis of blood samples.

For every experiment, immediately after collection, blood was transferred to EDTA coated tubes with 250 KIU/ml of Trasylol, kept on ice until centrifugation (10 minutes at 4°C and 3500 rpm) 1-1½ h after sampling. Plasma for glucose analysis was analyzed on the same day using the glucose oxidase method, with 10 μl plasma in 500 μl buffer (EBIO plus autoanalyzer and solution, Eppendorf). Plasma for insulin analysis was pipetted on dry ice and stored at −20°C until analysis. Porcine insulin (OGTT and IVGTT) was analyzed using an in-house two-site immunometric assay with monoclonal antibodies as catching and detecting antibodies and using purified porcine insulin for calibration of the assay. The minimal detectable concentration was 3.6 pM and the upper limit (with no sample dilution) was 1785 pM. Human insulin (mITT and hyperinsulinemic, isoglycemic clamp) concentration was analyzed using an in house LOCI sandwich immunoassay using two different monoclonal antibodies directed against rat insulin and human insulin, respectively. Cross-reactivity to pig insulin was 15%. The lower limit of quantification was 8 pM and the upper limit (with no sample dilution) was 3000 pM. C-peptide concentration was measured using a commercial RIA-kit from Linco Research, Inc., St. Charles, Missouri (Porcine C-peptide RIA kit cat. no. PCP-22K).

Calculations and statistical analysis.

Insulin sensitivity was evaluated from fasting values of glucose and insulin measured on the day of the IVGTT using the quantitative insulin sensitivity check index, \( \text{QUICKI} = \frac{1}{\log G_b + \log I_b} \) (21), where \( G_b \) and \( I_b \) are the basal (fasting) levels of glucose and insulin concentration, respectively.

From OGTT data, insulin sensitivity indices, previously developed in humans were calculated: \( \text{ISI}_{\text{comp}} = \frac{10000}{\sqrt{G_b I_b G_m I_m}} \), with \( G_m \) and \( I_m \) being the
mean glucose and insulin concentrations during OGTT (28) and oral glucose insulin sensitivity (OGIS; http://www.isib.cnr.it/bioing/ogis/home.html) (27). Oral glucose tolerance was determined as the Δ2h glucose value. Beta cell function was estimated with the insulinogenic index with insulin (ΔInsulin_{30min}/ΔGlucose_{30min}) and with C-peptide (ΔC-peptide_{30min}/ΔGlucose_{30min}), where Δ indicates the relative differences from fasting levels (41).

From the IVGTT, two insulin sensitivity indices were calculated: the minimal model derived insulin sensitivity index (8) and a new simpler index introduced here. This new index is defined as:

$$\frac{30 \times K_G}{\int_0^{30} I(t) dt}$$

$K_G$ is the classic intravenous glucose tolerance index: i.e., the negative slope of the linear regression of the logarithm of glucose versus time in the 25 min interval from 5 to 30 min. The term at the denominator is simply the area under the curve (AUC) of insulin concentration (which can be computed with the trapezoidal rule) from 0 to 30 min to take into account the delay in insulin action. The units of the new index are min^{-1}/(pmol/l), the same as those of the index from the minimal model. In order to compare these IVGTT indices with the glucose clamp, used as the reference gold standard value, it is necessary that the units are the same. The indices were therefore multiplied by the glucose distribution volume (Vd, ml/kg), as already done in humans (9), to obtain (ml/min)/(pmol/l) kg^{-1}. The new simplified index times Vd is termed S2 and from now on, we refer to S1 as the minimal model index times Vd. Vd from the minimal model derives from one of the estimated parameters. When the IVGTT is analyzed only until 30 min, Vd is calculated as the injected glucose dose divided by the glucose peak and body weight. Acute C-peptide response to glucose,
ACPR
subscript{G}, acute insulin response to glucose, AIR
subscript{G}, and mean glucose, G
subscript{m}, were calculated from the IVGTT as average C-peptide, insulin and glucose concentrations, respectively, from 1 to 5 min. Beta cell function was evaluated as both △ACPR
subscript{G}/△G
subscript{m} (beta cell sensitivity to glucose) and △AIR
subscript{G}/△G
subscript{m} (sensitivity of the post hepatic insulin release to glucose).

For the hyperinsulinemic, isoglycemic clamp the individual M values of the clamp (mg/min kg
superscript{-1}) were divided by the individual steady-state human insulin concentration in the interval 180-210 min and by the individual clamped glucose concentration in the same interval. Thus, the final units are also in this case (ml/min)/(pmol/l) kg
superscript{-1} and the resulting clamp index M/G/I is comparable to S1 and S2 for checking equivalence.

The mITT insulin sensitivity index was calculated as the negative slope of the logarithm of glucose in the interval 18-30 min versus time divided by the mean human insulin concentration in the same interval. This index was also multiplied by Vd (obtained from the minimal model) to obtain the final insulin sensitivity index (ISI
subscript{ITT}) in the same units of the other indices.

Reproducibility study.

QUICKI, S2 and △AIR
subscript{G} were calculated as described above, and furthermore the AUC
subscript{insulin(0-120)} and AUC
subscript{glucose(0-120)} were calculated from the trapezoid rule. Coefficients of variation (CV%) were calculated as SD/mean×100 of the two measurements for each pig. The median CV% for each parameter was used as a measure of reproducibility.
Statistical analysis.

Statistical analysis of the data was done in SAS (SAS® statistical software, v. 9.1 for Windows) using linear regression (Proc Reg in SAS). Statistical outliers and influential observations, identified as observations with a standard residual >3.0 or a Cook’s D-value>1, respectively, were excluded from the analysis. All indices were compared between pigs on LED and HED with student’s t-test. P-values ≤0.05 were considered statistically significant. Data and results are shown as mean±SEM.

Results

Characteristics of the minipigs and values of the glucose tolerance, beta cell function and insulin sensitivity indices are shown in Table 2 and regression coefficients are shown in Table 3. OGTT and IVGTT time vs. concentration curves are shown in Fig. 1 and 2, respectively, and the most relevant linear regressions are shown in Fig. 3.

The feeding of diets with different energy content to both male and female animals led to the desired quite wide range in body weight and consequently in insulin resistance or sensitivity. The mean daily food intake in grams was not different in the two groups (274±8 g in HED and 259±6 in LED, p=ns), but as expected the daily energy intake was significantly higher in the pigs on HED compared to the pigs on LED (1241±35 vs. 748±15, p<0.001).

Glucose tolerance calculated from the OGTT (Δ 2 h glucose) and IVGTT (K_G) correlated significantly (r=0.55, p<0.01), whereas no correlations were found between OGTT and IVGTT beta cell function indices.

The CV% for glucose and insulin concentrations during the steady state period of the clamp was 8 and 10%, respectively.
The new IVGTT derived insulin sensitivity index, S2, significantly correlated with M/G/I (r=0.54, p<0.05) showing a satisfactorily agreement between these two measurements. Minimal model S₁ on the contrary did not correlate with M/G/I, although it correlated significantly with S2. S2 furthermore correlated with ISI_{comp}, OGIS and ISI_{ITT} (Table 3, Fig. 1). In addition, ISI_{ITT} correlated with S₁, ISI_{comp} and OGIS, and the two OGTT indices correlated significantly with each other but not with M/G/I (Table 3).

Significantly lower insulin sensitivity was found in the pigs on HED compared to the pigs on LED both with M/G/I (1.0±0.1 vs.1.7±0.2, p<0.01), S2 (1.6±0.26 vs. 3.0±0.44, p<0.05) and ISI_{ITT} (1.8±0.2 vs. 3.0±0.2, p<0.001), but not with S₁, ISI_{comp}, OGIS or QUICKI (Table 2).

In the reproducibility study an IVGTT was performed twice in a separate group of animals, and the results of the two tests were compared. Body weights at the two test points were 24.2 ± 2.8 and 23.0 ± 2.2 kg (p<0.05). The CV% was 8.9 for basal insulin, 7.8 for basal glucose, 13.2 for S2, 13.9 for AUC_{insulin(0-120)} and 6.9 for AUC_{glucose(0-120)}.

Discussion.

Minipigs are being increasingly used in metabolic research (6, 12, 19, 25, 26, 31), underlining the necessity of valid methods to evaluate insulin sensitivity in this species. The hyperinsulinemic, iso- or euglycemic clamp is considered the “gold standard” for evaluation of insulin sensitivity in humans and presumably also in animal models (11, 30). However, due to the laboriousness of this test, several attempts have been made to introduce and validate new and simpler methods to assess insulin sensitivity in humans from both fasting measurements and dynamic
tests. Some of the most extensively used alternative indices are the IVGTT derived SI from the minimal model (8, 9, 37) and the OGTT derived ISI_{comp,} (28) and OGIS (27), but also indices from ITT have been exploited (4). All of these indices have been shown to correlate significantly with clamp measures in humans.

In addition to the evaluation of already existing methods, here a new and simple IVGTT-derived insulin sensitivity index, S2, was introduced and validated versus gold standard glucose clamp and other previously described indices from OGTT, IVGTT and mITT in Göttingen minipigs. The proposed S2 index in minipigs is based on the same assumptions of an IVGTT-derived insulin sensitivity index described in humans (14) and in mice (32). The time period 5-30 min used to calculate K_{G} was chosen because this period avoids the initial mixing phase of glucose and corresponds to the period with a log-linear fall in plasma glucose in these pigs. To calculate S2, K_{G} was divided by the AUC_{insulin} in the interval 0-30 minutes to take into account the possible delay in insulin action. The distribution volume, Vd, was included in order to obtain a measure totally comparable to clamp derived insulin sensitivity in terms of measurement units ((ml/min)/(pmol/l) kg^{-1}) (9). S2 significantly correlated with clamp-derived M/G/I, indicating its reliability for the evaluation of insulin sensitivity in pigs. Further support of the validity of this index was obtained, since, as expected, lower insulin sensitivity was found in the pigs on HED compared to the pigs on LED, both with the S2 index and the M/G/I index (Table 2). In addition, the reproducibility of S2 was satisfactory, being in the same range as that of IVGTT derived indices of insulin sensitivity in humans (13, 17, 39).

When compared with other widely used indices from OGTT, IVGTT and mITT, S2 also correlated significantly with SI, ISI_{comp}, OGIS and ISI_{ITT}, but not with QUICKI. Surprisingly, none of the other indices correlated significantly with clamp
insulin sensitivity. This may be due to the inherent differences between the various tests or to the existing differences between the tests in pigs and humans. The glucose clamp quantifies the insulin effect under experimental steady state conditions, and thus does not take into account the delay in insulin action. Depending on the insulin concentration, hepatic glucose production is inhibited to a varying degree; when insulin reaches high concentration levels primarily peripheral insulin sensitivity is estimated (30). In the present study, the insulin infusion of 2 mU/kg/min given during the clamp, was chosen to attain physiological insulin concentrations in the same range as the peak insulin values in the OGTT and IVGTT (414±32 vs. 410± 39 and 461± 91 pmol/l, respectively), making the three tests comparable in that respect. Furthermore, this dose is expected to lead to almost complete inhibition of hepatic glucose output in pigs (18), meaning that M/G/I represents mainly peripheral insulin sensitivity. QUICKI, on the other hand, has been shown to describe almost exclusively hepatic insulin sensitivity, whereas all of SI, S2 and ISI\(_{ITT}\) together with the OGTT derived indices presumably have both peripheral and hepatic components (1).

These differences can affect the degree of correlation, since individuals may have separate hepatic or peripheral insulin resistance, although they are usually well correlated (28, 30).

In addition, the dynamic tests (OGTT, IVGTT and mITT) are characterized by usually elevated levels of hyperglycemia and therefore glucose disappearance includes a component due to glucose-mediated glucose disposal, which can be quantified only with minimal model (2). The weight of this process may also affect the degree of correlation between the dynamic indices on one hand and M/G/I and QUICKI on the other hand. Furthermore, it probably explains why S2, SI and ISI\(_{ITT}\)
tend to overestimate insulin sensitivity from the clamp (fig. 1, A-C), despite the same units.

Moreover, the OGTT used in minipigs is not completely analogous to that used in humans, since the glucose is given as a mixed meal with a diet rich in fiber. This leads to a slower and more variable gastric emptying and absorption of glucose from the gastrointestinal channel (Fig. 1) and thereby to a smaller and more variable peak in plasma glucose. Thus, the OGTT in pigs comprises a weaker metabolic challenge compared to an IVGTT, which may make the OGTT less able to detect small differences in insulin sensitivity. Together with a possible incretin effect on insulin action (3, 40), this may explain the lack of significant difference in insulin sensitivity between the two diet groups and the lack of correlation to the clamp derived insulin sensitivity. Nonetheless, although probably only able to detect more prominent group differences in insulin sensitivity, the OGTT may still be useful in pigs for providing some information on glucose tolerance, insulin resistance and insulin secretion given the physiological conditions under which it is performed. The sub-maximal stimulus on the beta cells during OGTT compared IVGTT could, in combination with the incretin effect on insulin secretion, also explain the lack of correlation between beta cell function indices obtained by these two tests in pigs. However, since not the major focus of the present study, beta cell function indices were not compared to the gold standard hyperglycemic clamp.

From a methodological point of view, the insulin and glucose patterns during the IVGTT exhibited less dynamic profiles in pigs compared to humans and dogs (Fig. 2), where the minimal model has been more extensively exploited. Since the estimation of $S_I$ strongly depends upon the shape of the concentration patterns (16), it can be speculated that these qualitative differences may lead to an imprecise
minimal model estimate of insulin sensitivity in pigs and thus explain the lack of agreement between $S_i$ and clamp.

**Perspectives and significance.**

Currently, many different non-validated tests and indices are used to describe insulin sensitivity in (mini)pigs in situations where hyperinsulinemic clamp is not performed (which is the majority of the studies as it is now). This may make different studies, feeding regimens and effects of drug candidates difficult to compare. By introducing a quick, simple and validated intravenous method, we hope to make it "first choice” in settings where clamp is not performed. Thereby, the published studies in (mini)pigs would become more uniform and easier to compare, which would be a huge advantage. The new insulin sensitivity index, $S_2$, is simple to obtain from a short IVGTT and further it is the only index that appears to correlate with clamp in minipigs. The new index is based on general physiological principles not depending on the exact shape of the glucose and insulin curves, and is therefore likely to be applicable not only in Göttingen minipigs but also in other pigs and species. Indeed, this simplified test has already shown to be applicable in humans (14) and mice (32).

The applicability of $S_2$ in diabetic animals with low or no endogenous insulin secretion remains to be clarified, but the method may be further developed with exogenous insulin infusion like done with the insulin modified IVGTT in humans (35).

In conclusion, the new $S_2$ insulin sensitivity index, based on a short intravenous glucose tolerance test, significantly and exclusively correlated to the insulin sensitivity index from the hyperinsulinemic, isoglycemic clamp. The $S_2$ index is simple and useful for evaluation of insulin sensitivity in pigs.
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Figure legends.

**Figure 1.** Glucose (A) and insulin (B) concentrations during a 3 h OGTT in Göttingen minipigs on low energy (▲) and high energy (○) diet (n=10-13). Data are means ± SEM.

**Figure 2.** Glucose (A) and insulin (B) concentrations during a 2 h IVGTT in Göttingen minipigs on low energy (▲) and high energy (○) diet (n=12-13). Data are means ± SEM.

**Figure 3.** Relationship between different insulin sensitivity indices in Göttingen minipigs. A: M/GI vs. S2, B: M/G/I vs. SI, C: M/G/I vs. ISI_{ITT}, D: S2 vs. ISI_{ITT}, E: M/G/I vs. ISI_{comp}, F: S2 vs. ISI_{comp}, G: M/G/I vs. OGIS, H: S2 vs. OGIS. $P \leq 0.05$ significant, ns = not significant. ○: high energy diet, ▲: low energy diet.
Table legends.

**Table 1.** Proximate analysis of the two experimental minipig diets.

LED=low energy diet, HED=high energy diet

**Table 2.** Characteristics and metabolic parameters of the minipigs (mean±SEM). P<0.05 regarded significant, ns=not significant.

The experimental procedure and the formulas for calculating the metabolic parameters along with their meaning are reported in the Methods section.

**Table 3.** Regression coefficients (r) from linear regression of insulin sensitivity indices in Göttingen minipigs.

The indices were obtained with hyperinsulinemic, isoglycemic clamp, oral (OGTT) and intravenous (IVGTT) glucose tolerance tests and modified insulin tolerance test (mITT). n= 16-25. * p≤0.5, ** p≤0.01, *** p≤0.001. ns= not significant. See text for the meaning of the variables.
Table 1. Proximate analysis of the two experimental minipig diets.

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Figure 1. Glucose (A) and insulin (B) concentrations during a 3 h OGTT in Göttingen minipigs on low energy (△) and high energy (○) diet (n=10-13). Data are means ± SEM.
Figure 2. Glucose (A) and insulin (B) concentrations during a 2 h IVGTT in Göttingen minipigs on low energy (▲) and high energy (○) diet (n=12-13). Data are means ± SEM.
### Table 2. Characteristics and metabolic parameters of the minipigs (mean±SEM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>All animals</th>
<th>Low energy diet</th>
<th>High energy diet</th>
<th>High energy vs. low energy diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>-</td>
<td>11/14</td>
<td>6/7</td>
<td>5/7</td>
<td>-</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>-</td>
<td>15.1±0.5</td>
<td>13.1±0.2</td>
<td>17.4±0.4</td>
<td>≤0.001</td>
</tr>
<tr>
<td>M/G/I ((mL/min)/(pmol/L)kg⁻¹)</td>
<td>Clamp</td>
<td>1.4±0.1</td>
<td>1.7±0.2</td>
<td>1.0±0.1</td>
<td>≤0.05</td>
</tr>
<tr>
<td>S₁ ((mL/min)/(pmol/L)kg⁻¹)</td>
<td>IVGTT</td>
<td>2.0±0.3</td>
<td>2.5±0.4</td>
<td>1.4±0.3</td>
<td>ns</td>
</tr>
<tr>
<td>S₂ ((mL/min)/(pmol/L)kg⁻¹)</td>
<td>IVGTT</td>
<td>2.3±0.3</td>
<td>3.0±0.4</td>
<td>1.6±0.3</td>
<td>≤0.05</td>
</tr>
<tr>
<td>ISI_{comp}</td>
<td>OGTT</td>
<td>30.1±2.7</td>
<td>33.6±4.3</td>
<td>25.6±2.4</td>
<td>ns</td>
</tr>
<tr>
<td>OGIS (mL/min/kg)</td>
<td>OGTT</td>
<td>354±29</td>
<td>376±38</td>
<td>324±45</td>
<td>ns</td>
</tr>
<tr>
<td>ISI_{ITT} ((mL/min)/(pmol/L)kg⁻¹)</td>
<td>mIST</td>
<td>2.4±0.2</td>
<td>3.0±0.2</td>
<td>1.8±0.2</td>
<td>≤0.001</td>
</tr>
<tr>
<td>QUICKI_{IVGTT}</td>
<td>IVGTT</td>
<td>0.49±0.01</td>
<td>0.50±0.02</td>
<td>0.47±0.02</td>
<td>ns</td>
</tr>
<tr>
<td>K_{G} (min⁻¹)</td>
<td>IVGTT</td>
<td>4.2±0.3</td>
<td>4.7±0.3</td>
<td>3.6±0.4</td>
<td>ns</td>
</tr>
<tr>
<td>∆ACPR_{G}/∆M_{G} (pmol/mmol)</td>
<td>IVGTT</td>
<td>47.9±3.8</td>
<td>46.7±4.4</td>
<td>49.2±6.6</td>
<td>ns</td>
</tr>
<tr>
<td>∆AIR_{G}/∆M_{G} (pmol/mmol)</td>
<td>IVGTT</td>
<td>24.1±2.6</td>
<td>23.2±2.8</td>
<td>25.1±4.5</td>
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</tr>
<tr>
<td>∆2h glucose_{OGTT} (mM)</td>
<td>OGTT</td>
<td>1.7±0.2</td>
<td>1.5±0.2</td>
<td>2.0±0.4</td>
<td>ns</td>
</tr>
<tr>
<td>∆Insulin_{30min}/∆Glucose_{30min} (pmol/mmol)</td>
<td>OGTT</td>
<td>89.1±13.1</td>
<td>83.6±16.2</td>
<td>96.3±22.2</td>
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<tr>
<td>∆C-peptide_{30min}/∆Glucose_{30min} (pmol/mmol)</td>
<td>OGTT</td>
<td>186.0±22.4</td>
<td>179.5±28.1</td>
<td>194.3±38.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

The experimental procedure and the formulas for calculating the metabolic parameters along with their meaning are reported in the Methods section.
Figure 3. Relationship between different insulin sensitivity indices in Göttingen minipigs.
A: M/G/I vs. S2, B: M/G/I vs. S1, C: M/G/I vs. ISI_{ITT}, D: S2 vs. ISI_{ITT}, E: M/G/I vs. ISI_{comp},
F: S2 vs. ISI_{comp}, G: M/G/I vs. OGIS, H: S2 vs. OGIS. P ≤ 0.05 significant, ns = not significant. o: high energy diet, ▲: low energy diet.
Table 3. Regression coefficients (r) from linear regression of insulin sensitivity indices in Göttingen minipigs.

<table>
<thead>
<tr>
<th></th>
<th>M/G/I</th>
<th>S1</th>
<th>S2</th>
<th>ISI_{comp}</th>
<th>OGIS</th>
<th>ISI_{ITT}</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/G/I</td>
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<td>0.54*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>S1</td>
<td>0.81***</td>
<td>0.62**</td>
<td>ns</td>
<td>0.68***</td>
<td>ns</td>
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<td></td>
</tr>
<tr>
<td>S2</td>
<td>0.78***</td>
<td>0.48*</td>
<td>0.57**</td>
<td>0.42*</td>
<td>0.68***</td>
<td>0.42*</td>
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<td>0.43*</td>
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<tr>
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<td>ns</td>
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</tr>
<tr>
<td>QUICKI</td>
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</table>