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ARTICLE

An empirical index of insulin sensitivity from short IVGTT: validation against the minimal model and glucose clamp indices in patients with different clinical characteristics

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Abstract
Aims/hypothesis Minimal model analysis for insulin sensitivity has been validated against the glucose clamp and is an accepted method for estimating insulin sensitivity from IVGTT. However minimal model analysis requires a 3 h test and relevant expertise to run the mathematical model. The aim of this study was to suggest a simple predictor of minimal model analysis index using only 1 h IVGTT.
Methods We studied participants with different clinical characteristics who underwent 3 h regular (n=336) or insulin-modified (n=160) IVGTT, or 1 h IVGTT and euglycaemic–hyperinsulinaemic clamp (n=247). Measures of insulin sensitivity were insulin sensitivity index estimated by minimal model analysis (S_I) and the mean glucose infusion rate (clamp) (M). A calculated S_I (CS_I) predictor, $CS_I = \alpha \times K_G/(\Delta AUC_{INS}/T)$, was suggested, based on the calculation of the rate of glucose disappearance K_G and the suprabasal AUC of insulin concentration ΔAUC_{INS} over T=40 min. For all the participants, α was assumed equal to the regression line slope between $K_G/(\Delta AUC_{INS}/T)$ and S_I in control participants.
Results CS_I and S_I showed high correlation (R^2=0.68–0.96) and regression line slopes of approximately one in the majority of groups. CS_I tended to overestimate S_I in type 2 diabetic participants, but results were more reliable when CS_I was computed with insulin-modified rather than regular IVGTT. CS_I showed behaviours similar to S_I as regards relationships with BMI, acute insulin response and sex. CS_I showed good correlation with M (R^2=0.82).
Conclusions/interpretation A short test can achieve a good approximation of minimal model analysis and clamp insulin sensitivity. The importance of a method such as CS_I is that it allows analysis of IVGTT datasets with samples limited to 1 h.

Keywords Glucose tolerance · Insulin action · Insulin resistance · One hour intravenous glucose tolerance test

Abbreviations

AIR_G	Acute insulin response to glucose
AUC_{INS}	AUC of insulin concentration
CS_I	Calculated S_I
IGM_{CL}	Impaired glucose metabolism (participants subjected to clamp)
IGT	Impaired glucose tolerance
INSMOD	Insulin-modified 3 h frequently sampled IVGTT
M	Mean glucose infusion rate (clamp)
NGT	Normal glucose tolerance
NGT_{CL}	NGT participants subjected to clamp
S_I	Insulin sensitivity index estimated by minimal model analysis

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Introduction

Insulin sensitivity is paramount for characterising metabolic states. The glucose clamp is the experimental procedure yielding the gold standard measurement of this variable. Nonetheless, minimal model analysis of IVGTT data, i.e. insulin sensitivity index estimated by minimal model analysis (S_I), is also widely used to assess insulin sensitivity [1, 2]. However, despite some efforts to develop automatic procedures and lower the need for user intervention [3], the minimal model procedure requires sophisticated computer programming and relevant expertise to run the mathematical model properly. Furthermore, reliable results based on minimal model require many plasma insulin and glucose samples over a time interval of at least 3 h after glucose injection.

The aims of this study were: (1) to propose an index able to predict minimal model insulin sensitivity values based on direct calculations from easily measurable simple variables and not requiring complex mathematical models, while using IVGTT data limited to 1 h or less, as often happened before introduction of the minimal model [4]; and (2) to validate the new index against minimal model S_I and the glucose clamp, by assessing its performance in several groups of participants with different degree of glucose tolerance and specific clinical characteristics.

Methods

Participants analysed in this study are presented in the following sections. All participants gave their consent to the investigations, which were approved by the Local Ethics Committees.

Participants, 3 h regular IVGTT We analysed 336 participants partially studied in previous investigations [5–8]. Of these, 114 were control participants with normal glucose tolerance (NGT), 128 had impaired glucose tolerance (IGT) (22 of whom also had impaired fasting glucose) and 22 had type 2 diabetes (Table 1). The type 2 diabetes patients (diabetes duration 6.2±0.4 years) were diet-controlled; none of them were taking oral hypoglycaemic agents or insulin. We also analysed 52 participants with chronic renal disease from diabetic nephropathy, nine patients with hyperparathyroidism before and after parathyroidec-

Table 1 Main characteristics and insulin sensitivity in study groups

Study groups per protocol	Participant characteristics				Insulin sensitivity			
	n	Age (years)	BMI (kg/m^2)	G_b (mmol/l)	S_I ($\times10^{-4}$ min^{-1} [μU/ml]$^{-1}$)[a]	CS_I ($\times10^{-4}$ min^{-1} [μU/ml]$^{-1}$)[a]	*p* value	*M* (mg min^{-1} kg^{-1})[b]
	3 h regular IVGTT							
Control	114	34.4±1.6	23.6±0.5	4.6±0.05	5.55±0.25	5.81±0.28	0.22	–
IGT	128	42.8±1.4	27.9±0.6	4.7±0.09	2.58±0.17	2.68±0.20	0.49	–
Type 2 diabetes	22	41.2±4.8	23.8±0.5	6.3±0.20	2.31±0.29	4.68±0.69	0.0013	–
Renal disease	52	44.3±2.9	25.7±1.0	5.0±0.08	4.71±0.32	4.34±0.33	0.20	–
Hyperparathyroidism, pre	9	66.0±3.0	25.1±2.5	5.2±0.22	3.18±0.53	3.87±0.58	0.26	–
Hyperparathyroidism, post	9	66.0±3.0	24.4±2.3	5.2±0.26	5.34±0.67	6.66±0.96	0.043	–
Former type 1 diabetes	11	40.0±3.0	26.6±2.0	5.2±0.17	3.39±0.63	2.70±0.49	0.11	–
	3 h INSMOD							
Type 2 diabetes INSMOD	160	51.2±1.4	29.7±0.4	10.3±0.29	1.23±0.08	1.32±0.08	0.05	–
	1 h IVGTT and clamp							
NGT_{CL}	171	41.3±1.0	27.4±0.4	4.9±0.05	–	5.87±0.25	–	7.02±0.23
IGM_{CL}	55	46.1±1.6	29.1±0.7	5.4±0.08	–	4.16±0.39	–	6.08±0.32
Type 2 diabetes clamp	21	52.4±3.0	35.9±3.0	6.7±0.46	–	3.63±0.54	–	4.22±0.54

Values are mean±SE

[a] To convert values for S_I and CS_I to SI units ($\times10^{-4}$ min^{-1} [pmol/l]$^{-1}$), multiply by 0.1667

[b] To convert values for *M* to SI units (mmol min^{-1} kg^{-1}), multiply by 0.005551

G_b, basal glucose

tomy, and 11 patients who previously had type 1 diabetes (prior to kidney–pancreas transplantation) (Table 1). All participants underwent a regular 3 h frequently sampled IVGTT [9].

Type 2 diabetic participants, 3 h insulin-modified IVGTT We analysed from previous studies [10–12] 160 type 2 diabetic participants who had undergone an insulin-modified, 3 h, frequently sampled IVGTT (INSMOD) with exogenous intravenous infusion of insulin (0.03 or 0.05 U/kg) at 20 min [9] (Table 1). Some of these participants were under pharmacological treatment, with gemfibrozil [10], sulfonylurea or biguanide preparations [11].

Participants, 1 h IVGTT and clamp We analysed 247 participants from the Botnia study [13], the EUGENE2 study [14] and another study [15]. All these participants underwent IVGTT (for at least 1 h) and 2 h euglycaemic–hyperinsulinaemic glucose clamp. Among participants undergoing the clamp, 171 had NGT (NGT_{CL}), 55 had impaired glucose metabolism (IGM_{CL}), i.e. either impaired fasting glucose or IGT or both, and 21 had type 2 diabetes (Table 1). Seven participants in the type 2 diabetes clamp group had severe obesity and subsequently underwent bariatric surgery (here we only report data before surgery).

Calculation of insulin sensitivity In the participants with regular and INSMOD data, insulin sensitivity index was estimated by minimal model analysis (S_I). In the participants with the clamp, insulin sensitivity was calculated as the mean glucose infusion rate (M) over the last 40 min of the test. For all participants, we calculated a surrogate index of S_I, called calculated S_I (CS_I), with an expression similar to that originally proposed by Galvin et al. [16]. Justification of the difference between our approach and that of Galvin et al. [16] is discussed later. For the participants with regular IVGTT the expression for CS_I was:

$$CS_I = \alpha \frac{K_G}{\Delta AUC_{INS}/T} \quad (1)$$

where α is a constant (scaling factor), K_G is the rate of glucose disappearance (slope of log glucose), ΔAUC_{INS} is the AUC of insulin concentration above basal value and T is the time interval between 10 and 50 min (=40 min) when K_G and ΔAUC_{INS} are computed. Initial time interval was not zero to avoid possible confounding effects due to mixing. The α constant was assumed equal to the slope of the regression line between the factor $K_G/(\Delta AUC_{INS}/T)$ and S_I in the control group, i.e. α=0.276. This value was used to calculate CS_I in all the participants analysed in this study, including those undergoing INSMOD rather than regular IVGTT or clamp.

For the participants with INSMOD the expression for CS_I was:

$$CS_I = \alpha \times \left(\frac{\text{Average}(K_{G1}, K_{G2})}{\text{Average}(\Delta AUC_{INS1}, \Delta AUC_{INS2})/T} \right) \quad (2)$$

It is well known that the action of exogenous insulin on glucose disappearance is delayed [17], and hence we assumed a 5 min delay. Since insulin was injected at 20 min, K_{G1} and ΔAUC_{INS1} were computed between 10 and 25 min, whereas K_{G2} and ΔAUC_{INS2} were computed between 25 and 50 min.

Statistical analysis Relationships between S_I and CS_I were investigated by linear regression analysis with no intercept. Difference between the mean value of S_I and CS_I in each of the different groups of participants was assessed through the paired t test. The same test was used to assess difference in insulin sensitivity in the hyperparathyroidism group before and after surgery. Difference in the mean value of each index among different groups was assessed through ANOVA. Similarly, we analysed the relationship between CS_I and M by linear regression and used ANOVA to assess differences of both indices among different groups. Relationships between some variables were also investigated by accounting for measurement errors for both variables in the regression [18]. Normality of distributions was assessed before testing for possible differences in insulin sensitivity indices. In case of non-normal distributions, tests were performed on logarithmically transformed values (this applied to the majority of cases, except hyperparathyroidism and former type 1 diabetes groups). $p<0.05$ was considered statistically significant. Values are reported as mean±SE.

Results

Minimal model and CS_I analyses of regular IVGTT Strong correlation between S_I and CS_I was found in the following groups: control (R^2=0.89, $p<0.0001$, slope=1.00, 95% CI 0.93–1.07), IGT (R^2=0.79, $p<0.0001$, slope=0.97, 95% CI 0.89–1.06), renal disease (R^2=0.85, $p<0.0001$, slope=0.86, 95% CI 0.76-0.97), former type 1 diabetes patients (after kidney pancreas transplantation) (R^2=0.89, $p<0.0001$, slope=0.75, 95% CI 0.57-0.93) and hyperparathyroidism (R^2=0.83, $p<0.0001$, slope=1.09, 95% CI 0.68-1.49 before surgery; R^2=0.96, $p<0.0001$, slope=1.24, 95% CI 1.02–1.46 after surgery) (Fig. 1). In the type 2 diabetes group the correlation between S_I and CS_I, though weaker than in the other groups, was still significant (R^2=0.68, $p<0.0001$), despite the fact that CS_I overestimated S_I (slope=1.75, 95% CI 1.21-2.29). When the participants were considered all

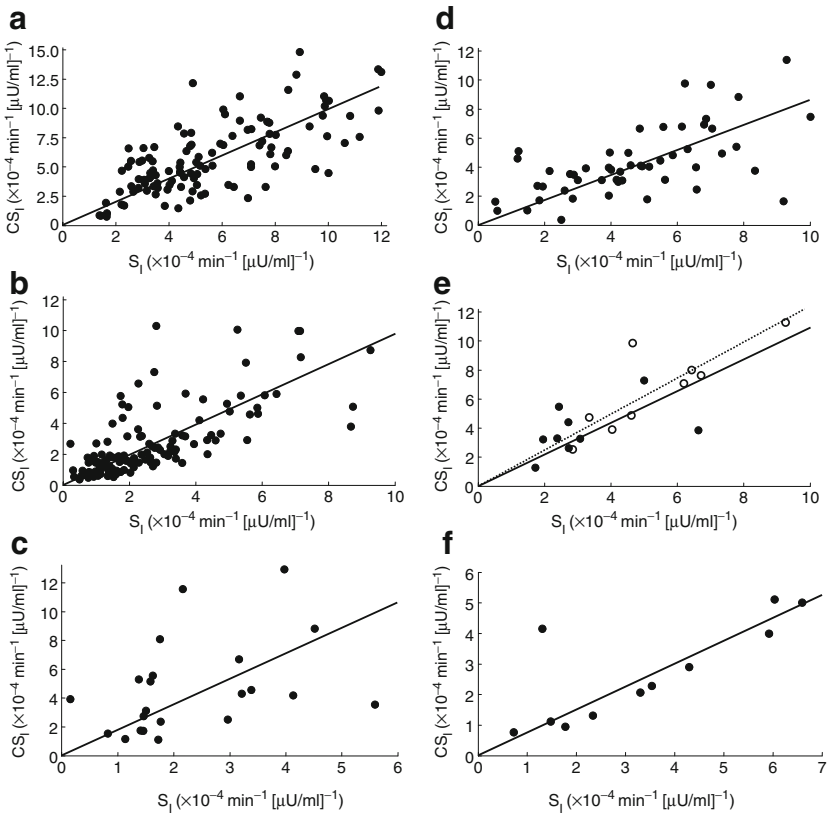

Fig. 1 Regression plots between insulin sensitivity from minimal model (S_I) and from CS_I index in (**a**) control participants, (**b**) participants with IGT, (**c**) type 2 diabetic participants (**d**) participants with chronic renal disease, (**e**) participants with hyperparathyroidism before and after parathyroidectomy and (**f**) former type 1 diabetic patients after kidney–pancreas transplantation. **e** White circles and dotted line are related to the condition after surgery. To convert values for S_I to SI units ($\times 10^{-4}$ min^{-1} [pmol/l]$^{-1}$), multiply by 0.1667

together, the correlation remained highly significant (R^2=0.84, p<0.0001, slope=0.99, 95% CI 0.94-1.04).

In each group, mean values of S_I and CS_I (Table 1) were not different except for the type 2 diabetes and hyperparathyroidism after surgery groups, which showed a slight difference as shown by p values (Table 1). Bland–Altman plot for all the participants (Fig. 2a) proved substantial equivalence between the two measurements. The absolute difference between S_I and CS_I in relation to the AUC of insulin in the time interval T (Fig. 2b) showed that only at low insulin levels did S_I and CS_I tend to diverge. ANOVA showed that S_I was different between control and all the other groups (p<0.03) except for the hyperparathyroidism after surgery group. Significant differences were also found in the renal disease vs IGT and type 2 diabetes groups (p<0.0001), and in the hyperparathyroidism after surgery vs IGT and type 2 diabetes groups (p<0.0006). Similar results were found for CS_I (p value range: p<0.0001 to p=0.0313), except for comparisons of type 2 diabetes with the other groups. It is worth noting the difference in insulin sensitivity between hyperparathyroidism before and after surgery: as expected, S_I was increased after surgery (p=0.021) (Table 1) and similar results were found with CS_I (p=0.008).

In the control group, we calculated the relationships between insulin sensitivity and acute insulin response to glucose (AIR_G) (mean insulin value above basal in the 0 to 8 min period of the IVGTT). Both S_I and CS_I showed with AIR_G a weak but significant nonlinear inverse relationship, which was better appreciated after performing linear regression analysis on logarithmically transformed values (R^2=0.19, p=0.0002 for S_I; R^2=0.09, p=0.0009 for CS_I) (Fig. 3). According to ordinary least-squares regression analysis, the relationship was not strictly hyperbolic, but it was similar with both indices. However, when the analysis was carried out through a regression method accounting for measurement errors in both variables, the relationship turned out to be hyperbolic, as the 95% CI for the slope included −1 for S_I (slope: −1.33, 95% CI −2.08, −0.59) and CS_I (slope: −1.25, 95% CI −2.15, −0.35).

In all the participants, we also analysed insulin sensitivity with respect to BMI. As expected, S_I showed an inverse relationship with BMI; in fact, after log-log transformation, a weak but significant linear regression was observed (R^2=0.19, p<0.0001), although the relationship was not hyperbolic (according to both regression methods). Similar results were found for CS_I (R^2=0.18, p<0.0001). Participants were then classified as lean or overweight according to their BMI (threshold 25 kg/m^2). Both S_I and CS_I showed significant differences in insulin sensitivity between the two groups (S_I=4.65±0.32×10^{-4} min^{-1} [μU/ml]$^{-1}$ lean; 3.09± 0.28 overweight; p=0.0003; CS_I=5.03±0.38×10^{-4} min^{-1}

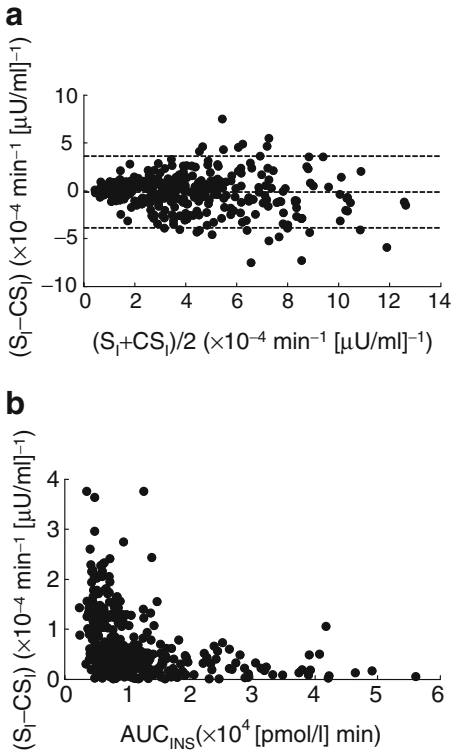

Fig. 2 Bland–Altman plot for S_I and CS_I in all the participants that underwent regular IVGTT (**a**) and absolute difference between S_I and CS_I in relation to the insulin AUC (**b**). Dotted lines (**a**) represent mean (middle line), 1.96 SD (top) and −1.96 SD (bottom). To convert values for S_I and CS_I to SI units ($\times 10^{-4}$ min^{-1} $[pmol/l]^{-1}$), multiply by 0.1667

$[\mu U/ml]^{-1}$ lean; 2.99±0.28 overweight; $p<0.0001$; to convert values for S_I and CS_I to SI units ($\times 10^{-4}$ min^{-1} $[pmol/l]^{-1}$), multiply by 0.1667).

We also studied possible differences in insulin sensitivity due to sex: neither S_I nor CS_I were different: S_I=3.87±0.17×10^{-4} min^{-1} $(\mu U/ml)^{-1}$ men; 4.09±0.28 women; $p>0.4$; CS_I=4.11±0.20×10^{-4} min^{-1} $(\mu U/ml)^{-1}$ men; 4.77±0.33 women; $p>0.07$.

Minimal model and CS_I analyses of insulin-modified IVGTT In the type 2 diabetes INSMOD group, S_I and CS_I showed strong significant correlation, with the slope of the regression line virtually equal to 1 (R^2=0.85, $p<0.0001$, slope=0.96, 95% CI 0.89-1.02) (Fig. 4a). Bland–Altman plot showed that only a few samples were outside the limits for equivalence (Fig. 4b). The paired t test showed a borderline p value (Table 1). We also classified the participants as obese or non-obese. Since BMI was quite high on average (BMI=29.7±0.4 kg/m^2), we assumed 27.5 as threshold between the two groups. As expected, S_I was higher in the non-obese group, with similar results found for CS_I (S_I=1.77±0.18×10^{-4} min^{-1} $[\mu U/ml]^{-1}$ non-obese; 0.94±0.08 obese; $p<0.0001$; CS_I=1.73±0.17×10^{-4} min^{-1} $[\mu U/ml]^{-1}$ non-obese; 1.10±0.08 obese; p=0.0002). Neither S_I nor CS_I were different between men and women (S_I=1.19±0.11×10^{-4} min^{-1} $[\mu U/ml]^{-1}$ men; 1.04±0.14 women; $p>0.5$; CS_I=1.30±0.10×10^{-4} min^{-1} $[\mu U/ml]^{-1}$ men; 1.03±0.11 women; $p>0.2$).

Glucose clamp and CS_I analyses In all the participants grouped together, M and CS_I showed good correlation (R^2=0.82, $p<0.0001$) (Fig. 5a). When analysing the participants divided according to glucose tolerance, correlation remained significant (NGT_{CL}: R^2=0.84, $p<0.0001$; IGM_{CL}: R^2=0.74, $p<0.0001$; type 2 diabetes clamp: R^2=0.81, $p<0.0001$) (Fig. 5b). M (Table 1) was different in each group ($p<0.03$). Similar differences ($p<0.002$) were found with CS_I (Table 1) except in the IGM_{CL} and type 2 diabetes clamp groups, where statistical significance was not reached. M was higher in lean than in overweight participants (8.72±0.33 and 5.58±0.18 mg min^{-1} kg^{-1}, $p<0.0001$; to convert values for M to SI units [mmol min^{-1} kg^{-1}], multiply by 0.005551), as was the case for CS_I (7.56±0.38 and 4.26±0.20×10^{-4} min^{-1} $[\mu U/ml]^{-1}$, $p<0.0001$). As regards possible differences related to sex, neither M nor CS_I showed any difference ($p>0.07$). In a subgroup of participants, we corrected M for the steady-

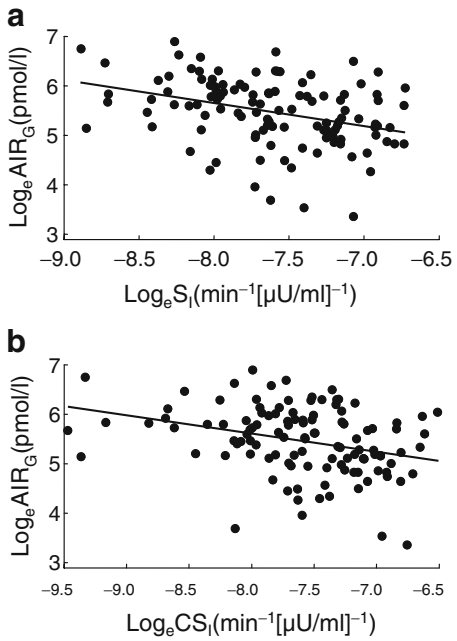

Fig. 3 Regression plot between S_I and AIR_G (**a**), and CS_I and AIR_G (**b**) in the control participants that underwent regular IVGTT. Variables are logarithmically transformed (log_e). Regression line equations were: for S_I $y = 1.98 - 0.46x$ (95% CI for intercept and slope 0.19, 3.77 and −0.69, −0.22, respectively); for CS_I $y = 2.80 - 0.35x$ (95% CI for intercept and slope 1.24, 4.36 and −0.56, −0.15, respectively). To convert values for S_I and CS_I to SI units ($\times 10^{-4}$ min^{-1} $[pmol/l]^{-1}$), multiply by 0.1667

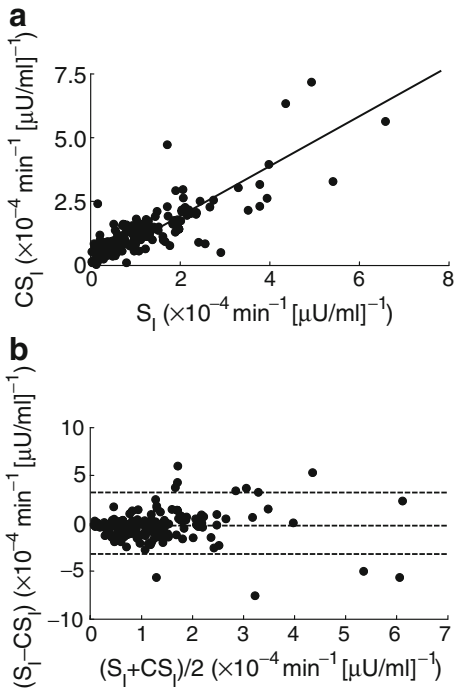

Fig. 4 Regression plot between S_I and CS_I in type 2 diabetic participants that underwent INSMOD (**a**) and Bland–Altman plot for S_I and CS_I (**b**). Dotted lines (**b**) represent mean (middle line), 1.96 SD (top) and −1.96 SD (bottom). To convert values for S_I and CS_I to SI units ($\times 10^{-4}$ min^{-1} [pmol/l]$^{-1}$), multiply by 0.1667

state insulin level, but results did not change significantly (not shown). It is worth noting that in the small group of type 2 diabetes clamp participants with severe obesity, 3 h IVGTT data were available, thus S_I was computed. As expected, we found agreement between S_I and M, with regression coefficient value (R^2=0.63, p=0.018) similar to those observed between CS_I and M, as reported above. In this specific subgroup, CS_I showed a very strong relationship with M (R^2=0.95, p<0.0001).

Discussion

The simple index of insulin sensitivity introduced and validated here (CS_I) was revealed to be a good surrogate of that from the well accepted and widely used minimal model (S_I). To our knowledge, only the study of Galvin et al. [16] suggested a simple index for the assessment of insulin sensitivity from IVGTT limited to 1 h. CS_I reflects similar concepts, i.e. the quantification of glucose disappearance rate per changes of insulin, but it overcomes some limitations of that study. In fact, Galvin et al. [16] studied the correlation of their index with S_I (and also with insulin sensitivity by the glucose clamp), but they did not seek to obtain indices really comparable, their units being different. In addition, they did not present any strategy to correct their index and make it comparable with S_I derived from insulin-modified IVGTT. In contrast, CS_I includes a time (T) factor (see Eq. 1) yielding the same units as S_I and was adapted to be used also with the insulin-modified test (Eq. 2). Furthermore, in Galvin et al. [16], the slopes of the regression lines were far from one and different in every group. Moreover, only small groups of participants were studied (with no diabetic patients) and it was not shown whether their index has abilities, similar to S_I, to discriminate between groups or clinical conditions with different degrees of insulin resistance. The Galvin index [16] was then used by Anderson et al. [19], but with essentially the same limitations, which probably prevented its diffusion. Prior to this study, we used calculations similar to those for CS_I to compute a sensitivity index in mice [20], although not with exactly the same formula and without comparison with the clamp.

After correcting our index with a factor derived from regression analysis of the control group (quite a large group, with a wide range of insulin sensitivity), several other groups of participants with different degrees of

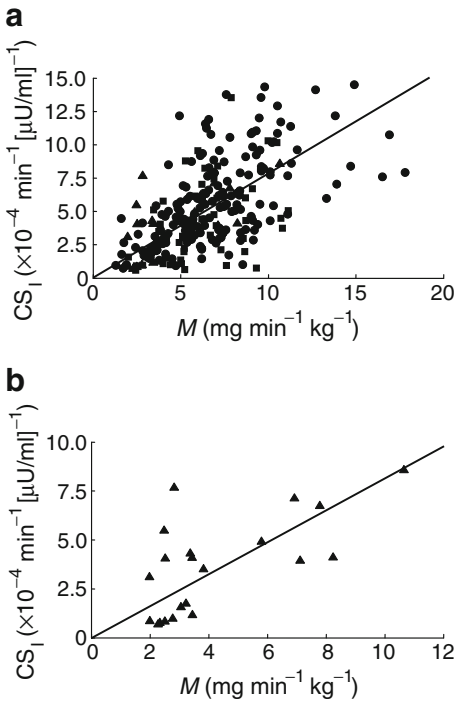

Fig. 5 Regression plot between insulin sensitivity from the clamp (M) and from the empirical index (CS_I) in (**a**) NGT_{CL} participants (circles), IGM_{CL} participants (squares) and type 2 diabetes clamp group participants (triangles). The regression line equation was $y = 7.82 \times 10^{-5}x$ (95% CI 7.36, 8.27×10^{-5}). Regression plot as above (**a**) for type 2 diabetes clamp group alone (**b**). Regression line equation: $y = 8.08 \times 10^{-5}x$ (95% CI 6.27, 9.89×10^{-5}). To convert values for M to SI units (mmol min^{-1} kg^{-1}), multiply by 0.005551

glucose tolerance and heterogeneous clinical characteristics were analysed. In the majority of groups, we found a good correlation between S_I and CS_I, and also CS_I values similar to S_I, as mirrored both by the slope of the regression lines, which were not (or only slightly) different from 1 (see 95% CI), and by the not significantly different mean values.

The correction factor α included in the CS_I expression was introduced to scale the values of our new index to those calculated with the minimal model. Thus, the interpretation of results obtained by CS_I will be facilitated, given the previous wide experience with S_I. This correction factor does not have a specific physiological meaning, similarly to the variables included in other empirical methods for the calculation of insulin sensitivity, such as HOMA-insulin resistance (IR) [21] or Stumvoll's index [22]. The relevant aspect of the scaling operation was that the same value of the correction factor (α=0.276) was proved to be appropriate in every group of participants (except type 2 diabetes, as discussed below). In fact, all the results were obtained by using the same correction factor in each group that underwent the regular IVGTT. The same α value was also proved correct in those groups of participants who underwent INSMOD (type 2 diabetes INSMOD) or the clamp (NGT_{CL}, IGM_{CL}, type 2 diabetes clamp).

The comparison between S_I and CS_I was not completely satisfactory in type 2 diabetes (regular IVGTT). The fact that in situations of high insulin resistance CS_I tended to overestimate S_I is an important issue and should be discussed within the frame of basic questions, such as: how reliable is a low S_I? This has been much debated among investigators using IVGTT [23, 24]. Thus, we acknowledge that, in situations of low insulin sensitivity, CS_I may suffer from inaccuracy, but S_I may also exhibit inaccuracy in those conditions [24, 25]. As regards our data, insulin levels in the type 2 diabetes group were usually low, but tended to remain higher than the fasting value: i.e. insulin levels did not return to the basal value during the whole 3 h IVGTT time interval. Thus, in the minimal model approach, the analysis of the last part of the IVGTT tended to decrease the S_I value. Since the last part of the complete test is not accounted for by CS_I, some discrepancy between the two indices may occur. On the other hand, the finding that in the majority of groups CS_I behaves similarly to S_I suggests that the information provided by the last part of the IVGTT is usually consistent with that provided by the first part, where CS_I is calculated.

Due to the unsatisfactory results in the type 2 diabetes group, we adapted the CS_I expression to make it usable with data from the insulin-modified IVGTT as recommended in conditions of poor insulin response [26]. We analysed a large group of type 2 diabetic patients subjected to INSMOD where, as expected, CS_I and S_I showed low values of insulin sensitivity. They also exhibited a strong correlation with regression slope almost identical with 1, confirming that when dealing with low insulin sensitivity it is recommended to carry out the insulin-modified test even with the short 1 h protocol. We also analysed 208 insulin-modified IVGTT from 146 women with a history of gestational diabetes, who were non-diabetic at the time of examination [27]. We found strong relationship between S_I and CS_I, with R^2=0.93 and slope of the regression almost equal to 1 (not shown). However, in non-diabetic participants the regular IVGTT has proven adequate for calculating CS_I with sufficient accuracy; hence the insulin-modified protocol is not strictly necessary in these participants. It should be noted that other possible expressions were tested for the calculation of CS_I with the insulin-modified IVGTT, such as the average between $(\alpha \times K_{G1})/(\Delta AUC_{INS1}/T1)$ and $(\alpha \times K_{G2})/(\Delta AUC_{INS2}/T2)$, with $T1$=15 and $T2$=25 min, and also the second expression alone (i.e. only post-injection information). However, the best results in diabetic and non-diabetic participants were obtained by combining pre- and post-injection information as in Equation (2).

CS_I was able to reproduce known findings related to insulin sensitivity. The existence of nonlinear inverse (hyperbolic) relationship between insulin sensitivity and insulin release was postulated some years ago [28] and several subsequent studies [29] have confirmed this finding, although it has recently been suggested that the hyperbola may not be evident in some groups of participants [30–32]. Our control group exhibited a weak, but still significant inverse relationship between insulin sensitivity and AIR_G. According to traditional regression analysis, the relationship was not strictly hyperbolic, but when a more refined regression model was used the hyperbolic relationship emerged. It is worth noting that S_I and CS_I provided similar results in both cases. Insulin sensitivity was higher in lean than in overweight or obese participants with both indices, which also showed a nonlinear inverse relationship (though weak) with BMI, in agreement with previous studies [33]. As regards the effect of sex on insulin sensitivity, results from S_I and CS_I were again similar and in agreement with previous studies [34].

Even though a good agreement was found between S_I and CS_I, we aimed to validate CS_I against the measurement obtained from the glucose clamp. CS_I exhibited a good degree of correlation with M and a similar ability to discriminate between participants with different glucose tolerance, as well as between lean and overweight participants. This agreement with the clamp further strengthened the ability of CS_I to describe insulin sensitivity in different metabolic conditions.

In this study we included three groups of type 2 diabetic patients. As regards the type 2 diabetes and type 2 diabetes INSMOD groups, it must be noted (Table 1) that both S_I and CS_I were higher in the former than the latter (p<0.0001 by ANOVA). This possible inconsistency warrants further

comment. First, it cannot be excluded that this difference in insulin sensitivity was real, since type 2 diabetic populations may be significantly heterogeneous [35]. On the other hand, as already pointed out, S_I may be inaccurate in participants with low insulin values, and CS_I exhibits similar limitations in those conditions. Another confounding factor may be the fact that the type 2 diabetes and type 2 diabetes INSMOD groups were studied in different laboratories, probably using different insulin assays: this remains a problem known to be a possible source of error [36]. In any case, we believe that the lower insulin sensitivity in the type 2 diabetes INSMOD than in the type 2 diabetes group may not be an artefact: in fact, HOMA-IR was also clearly higher in the former (7.85 vs 3.47 [non-dimensional], $p<0.007$), possibly also due the much higher BMI (Table 1). Similar comments hold for the significant difference in CS_I values ($p<0.0001$) between IGT and IGM_{CL}.

In conclusion, although the minimal model analysis remains the reference method to assess insulin sensitivity from the 3 h IVGTT, the proposed simple, empirical index CS_I generally proved to be a reliable index. In the condition of low insulin sensitivity, quite common in type 2 diabetes, analysis of insulin-modified rather than regular IVGTT data should be performed to obtain more reliable estimations, although it is known that in such conditions the assessment of insulin sensitivity becomes intrinsically more uncertain and possibly inaccurate. The great advantage of CS_I is that it allows assessment of insulin sensitivity from IVGTT data limited to 1 h, which cannot be analysed with the minimal model. The possibility of analysing less expensive short IVGTTs makes performance of the test easier and less of a burden for participants and investigators, allowing in larger populations the simultaneous assessment of insulin sensitivity and beta cell function (e.g. AIR_G variable) with a simple approach. CS_I also allows retrospective studies on all the short IVGTTs commonly performed before the introduction of the minimal model.

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