COMPARISON OF INSULIN SENSITIVITY INDICES CALCULATED FROM STANDARD 3-SAMPLED AND FREQUENTLY SAMPLED ORAL GLUCOSE TOLERANCE TEST

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Objective. Three-sampled oral glucose tolerance test is the most frequently used method for evaluation of impairment of glucose homeostasis in daily clinical practice. The aim of this study was to answer the question if insulin sensitivity indices (ISI) calculated from standard 3-sampled oral glucose tolerance test (3SoGTT) provide adequate information compared to the outcome when calculated from frequently sampled oral glucose tolerance test (FSoGTT).

Methods. A total of 73 subjects (aged 17-59 years, BMI 17.9- 41.8 kg/m^2) underwent a standard frequently sampled oral glucose tolerance test (FSoGTT). Selected indices of insulin sensitivity were calculated using plasma glucose and insulin concentrations from FSoGTT and from samples obtained in 0, 60 and 120 min of the oGTT (3SoGTT). Areas under the peripheral concentration curves of insulin and glucose (AUCi, AUCg) from both approaches were compared.

Results. Insulin sensitivity calculated from 3SoGTT was significantly higher compared to the sensitivity calculated from FSoGTT expressed as insulin sensitivity indices ISI Cederholm ($ISI_{(Ced)}$) and ISI Matsuda ($ISI_{(Mat)}$), $p<0.001$ and $p<0.05$, respectively. There was a difference in AUCg between values estimated from 3SoGTT and FSoGTT ($p<0.05$). These differences nearly disappeared when the BMI groups (normal weight and overweight/obese) were evaluated separately. No differences were found in AUCi and the AUCg : AUCi ratio between two approaches.

Conclusions. It might be supposed that on using 3SoGTT the $ISI_{(Mat)}$ provides greater objectivity in assessing insulin sensitivity than $ISI_{(Ced)}$. Although insulin sensitivity is overestimated when calculated from 3SoGTT, the approach is still valuable for identifying subjects with insulin resistance.

Key words: Glucose - Insulin - Insulin sensitivity indices - Glucose tolerance

The evaluation of insulin sensitivity is being supposed an important predictor of diabetes mellitus type 2, cardiovascular diseases and metabolic syndrome (WHO 1999). In the pre-disease stage, the decreased insulin sensitivity is compensated by increased secretion of insulin, which maintains normal fasting glucose and glucose disposal after a glucose load. Clamp methods (DEFRONZO et al. 1979) considered the "gold standard" in estimation of insulin sensitivity and beta cell secretory function are expensive, inconvenient and therefore not applicable in daily clinical practice. In spite of that, detection of subjects with increased risk of metabolic or cardiovascular disturbances is of high priority in the strategy of prevention.

The main demand in clinical practice and epidemiological studies is to obtain maximum information from a minimum of output data. The oral glucose tolerance test (oGTT) recommended by WHO (ALBERTI and ZIMMET 1998) is a widely used method for estimation of whole-body glucose tolerance *in vivo* (MATSUDA and DEFRONZO 1999). In several studies, insulin sensitivity indices based on fasting or post-load plasma glucose and insulin concentrations during oGTT have been established as less demanding, practical and valuable indicators of insulin resistance. Oral glucose tolerance test takes into account physiological processes in the human body. After glucose ingestion, there are several important fac-

tors affecting plasma glucose concentration such as the amount of glucose administered (Myers et al. 1991), the way of glucose administration (Ishida et al. 1983), differences in gastric emptying and glucose absorption (Horowitz et al. 1993, Rayner et al. 2001, Eliasson et al. 1995), hormonal factors such as insulin and glucagon concentrations in the portal system (Myers et al. 1991). Considering the interindividual variability of these processes, a high interindividual heterogeneity in glucose concentrations and insulin response during an oGTT can be expected (Fig. 1).

The question remains, however, if standard 3-sampled oGTT sampled in 0, 60 and 120 min (3SoGTT) adequately detects insulin resistance as compared to the frequently sampled oral glucose tolerance test (FSoGTT). The aim of the study was to assess whether insulin sensitivity indices and areas under the curve (0-120 min) for glucose (AUCg) and for insulin (AUCi) estimated from 3SoGTT provide adequate results compared to values obtained during FSoGTT.

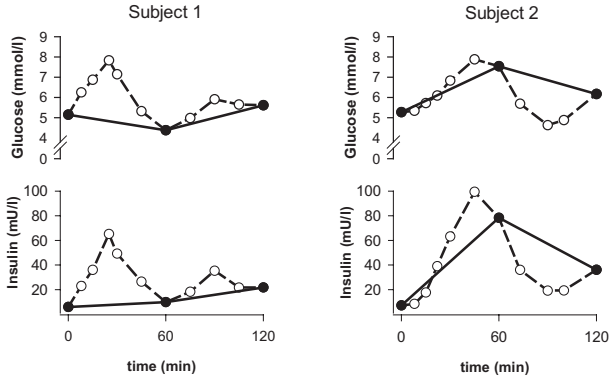

Fig.1 Glycemic and insulinemic curve during oGTT of 2 subjects with normal glucose tolerance. Standard 3SoGTT - solid line, FSoGTT - dashed line.

Subjects and Methods

Subjects. Seventy-three healthy volunteers (50 males and 23 females, aged 17-59 years) participated in this study. Exclusion criteria included previous knowledge of glucose metabolism alterations, the use of medications known to alter insulin secretion or action and presence of hepatic or endocrine diseases. The subjects were instructed to abstain from the use of alcohol, caffeine, tobacco and strong physical activity for 24 h and to fast for 12 h before investigation. After explanation of the procedure, written voluntary consent was obtained from all subjects. The Ethical Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences has approved the project design.

Test protocol. The investigation started at 8:00 AM at the Institute of Experimental Endocrinology, Slovak Academy of Sciences. An indwelling catheter (Surflo-W Terumo, Belgium) was inserted into an antecubital vein for blood sampling. After a resting period of at least 30 min in a comfortable armchair, blood sample for basal values was taken. Thereafter the subjects ingested 75 g of anhydrous glucose diluted in 250 ml water within 1-3 minutes. Blood samples for glucose and insulin estimations were obtained 8, 15, 22, 30, 45, 60, 90, 105 and 120 minutes after the complete glucose solution had been swallowed.

Analytical techniques. After centrifugation at 4°C and separation, aliquots of plasma were stored at -20°C until assayed. The plasma glucose concentration was measured using a glucose oxidase method (Hitachi, Japan). Plasma insulin concentration was measured by commercial IRMA kit (Immunotech S.A., France).

Insulin sensitivity indices. The following parameters were calculated:

1. Insulin resistance was estimated using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), using the formula (Matthews et al. 1985):
   HOMA-IR = (fasting insulin [mIU/l] x fasting glucose [mmol/l])/22.5;
2. Index of peripheral insulin sensitivity as proposed by Cederholm and Wibell (1990):
   $$ISI_{(Ced)} = \frac{75000 + (G_0 - G_{120}) \times 1.15 \times 180 \times 0.19 \times BW}{120 \times G_{mean} \times \log(I_{mean})};$$
3. Composite whole body insulin sensitivity index ($ISI_{(Mat)}$) as proposed by Matsuda and DeFronzo (1999):
   $$ISI_{(Mat)} = \frac{10000}{\sqrt{G_0 \times I_0 \times G_{mean} \times I_{mean}}};$$
4. The areas under the curve (0-120 min) for glucose (AUCg) and insulin (AUCi) and their ratio (AUCi : AUCg) were also estimated from 3SoGTT and from FSoGTT. The areas under the curve were calculated using the trapezoidal rule.

Statistical evaluation. The results were expressed as the mean ± standard error (SE). Mann-Whitney rank sum test was used for comparison of FSoGTT and 3SoGTT-derived insulin sensitivity indices and for AUCs using the Sigma Stat 2.0 program (Jandel Scientific, USA).

Results

The subjects were divided into two groups: normal weight (BMI <25 kg/m^2) and overweight/obese subjects (BMI ≥25 kg/m^2). Table 1 shows clinical characteristics of the study subjects. According to the diagnostic criteria of the AMERICAN DIABETES ASSOCIATION (2004), 59 (80.8 %) subjects in our study had normal glucose tolerance (NGT), 3 (4.1%) had impaired glucose tolerance (IGT), 7 (9.6 %) had impaired fasting glucose (IFG) and 4 (5.5 %) had combined IGT and IFG, none had diabetes mellitus. The insulin resistance index HOMA-IR was, as expected, significantly higher in overweight/obese subjects than in normal weight subjects ($p<0.001$).

Table 2 shows the values of insulin sensitivity indices ($ISI_{(Ced)}$, $ISI_{(Mat)}$), AUCg, AUCi and AUCi/AUCg obtained from 3SoGTT and from FSoGTT. There was a difference in AUCg between values estimated from 3SoGTT and FSoGTT ($p<0.05$). However, this difference could be observed only in normal weight subjects ($p<0.05$), but not in overweight/obese subjects ($p=0.406$). No differences were found in AUCi and in the AUCg : AUCi ratio between two approaches, either in the two BMI groups together or taken separately. Insulin sensitivity calculated from 3SoGTT was significantly higher as compared to the sensitivity calculated from FSoGTT, expressed as $ISI_{(Ced)}$ and $ISI_{(Mat)}$, $p<0.001$ and $p<0.05$, respectively. However when the BMI groups were evaluated separately, the differences remained significant only in the normal weight group.

Discussion

For the assessing of insulin resistance in epidemiological studies, convenient approaches such as insulin sensitivity indices are available. Generally, it might be supposed that the results of a dynamic test will be more accurate with a larger number of data used for the calculation. In a large prospective study, HANLEY et al. (2003) found the Gutt index (GUTT et al. 2000) of the best ability to predict type 2 diabetes. However, only those indices which were calculated either with the use of only fasting or fasting plus oGTT-derived 120 min glucose and insulin concentrations were included in the final evaluation. It can be assumed that more correct information should be obtained when results are calculated from a large number of samples taken in frequent intervals of oGTT (Fig. 1).

Actualy, one of the main questions we raised was whether the number of samples obtained from the standard oGTT markedly influences the result of the calculations of insulin sensitivity indices. Thus, we compared the insulin sensitivity indices as calculated from values obtained from frequently sampled oGTT with these from 3-sampled oGTT insulin sensitivity indices. The presented results indicated that both calculated indices, $ISI_{(Ced)}$ and $ISI_{(Mat)}$ were significantly higher when calculated from 3SoGTT than from FSoGTT. However, a lower significance level ($p<0.05$) was found between 3SoGTT- and FSoGTT-derived $ISI_{(Mat)}$ results in normal weight subjects, while no significant differences appeared in overweight/obese subjects. A better correlation with the hyperinsulinemic euglycemic clamp was found in $ISI_{(Mat)}$ compared to $ISI_{(Ced)}$ (MATSUDA and DEFRONZO 1999, STUMVOLL et al. 2000). These results supported the assumption that on using 3SoGTT, $ISI_{(Mat)}$ provides greater objectivity that in assessing insulin sensitivity than does $ISI_{(Ced)}$.

Table 1.
Clinical characteristics of subjects studied.
Data are expresed as the mean ± SEM.

	All subjects (all) (n=73)	Normal weight (nw) (n=48)	Overweight/obese (ob) (n=25)
Age (years)	26.7 ± 1.1	22.8 ± 0.6	34.1 ± 2.6
Gender (M/F)	50/23	37/11	13/12
NGT/IFG/IGT/IFG+IGT/DM	59/7/3/4/0	45/2/1/0/0	14/5/2/4/0
BMI (kg/m^2)	24.7 ± 0.6	21.9 ± 0.3	30.0 ± 0.8
Fasting glucose (mmol/l)	5.20 ± 0.06	5.08 ± 0.05	5.43 ± 0.14
Fasting insulin (mIU/l)	9.77 ± 0.90	7.64 ± 0.81	13.86 ± 1.89
HOMA - IR	2.34 ± 0.24	1.76 ± 0.20	3.46 ± 0.54
2-h glucose (mmol/l)	5.49 ± 0.18	5.17 ± 0.17	6.11 ± 0.39
2-h insulin (mIU/l)	36.6 ± 3.5	30.1 ± 3.4	49.0 ± 7.5

Table 2.
Parameters of insulin sensitivity and AUC calculated from 3SoGTT and FSoGTT for all subjects (all) and separately for normal weight (nw) and overweight/obese (ob) groups. Data are expresed as the mean ± SEM.

	3SoGTT	FSoGTT	p
ISI Cederholm (all)	77.8 ± 3.6	61.1 ± 2.1	<0.001
ISI Cederholm (nw)	85.5 ± 4.4	66.3 ± 2.5	0.002
ISI Cederholm (ob)	63.4 ± 4.9	50.9 ± 2.7	NS
ISI Matsuda (all)	7.85 ± 0.58	6.09 ± 0.40	0.045
ISI Matsuda (nw)	9.34 ± 0.74	7.23 ± 0.50	NS
ISI Matsuda (ob)	5.01 ± 0.61	3.91 ± 0.37	NS
AUCins (all)	5.21 ± 0.35	5.51± 0.40	NS
AUCins (nw)	4.29 ± 0.32	4.27 ± 0.40	NS
AUCins (ob)	6.97 ± 0.69	7.90 ± 0.69	NS
AUCglu (all)	0.75 ± 0.02	0.66 ± 0.03	0.041
AUCglu (nw)	0.71 ± 0.02	0.59 ± 0.04	0.018
AUCglu (ob)	0.83 ± 0.04	0.78 ± 0.05	NS
AUCins/AUCglu (all)	6.70 ± 0.36	8.63 ± 0.63	NS
AUCins/AUCglu (nw)	5.90 ± 0.38	7.38 ± 0.53	NS
AUCins/AUCglu (ob)	8.23 ± 0.67	11.05 ± 1.43	NS

There is no doubt that the FSoGTT-derived glycemic curve describes more precisely the situation after oral glucose load than the 3SoGTT-derived curve. Also AUCG calculated from the FSoGTT were significantly higher than those calculated only from 3SoGTT, predominantly in normal weight subjects. Calculation of AUCG only from 3 points of an oGTT cannot be recommended.

Interestingly, there were no significant differences in insulin response between the two calculation approaches. Mean glucose concentration, but not insulin concentration, can be considered the most important input variable in both indices responsible for the differences between the 3SoGTT and FSoGTT.

Three-sampled oGTT-derived $ISI_{(Ced)}$ is significantly higher than the FSoGTT-derived one, particularly in normal weight subjects. That means that a subject considered "insulin sensitive" on using 3SoGTT-derived $ISI_{(Ced)}$ might be significantly less insulin sensitive when $ISI_{(Ced)}$ is calculated from FSoGTT. This limitation could be observed for $ISI_{(Ced)}$ only in normal weight subjects but not in overweight/obese subjects, while for $ISI_{(Mat)}$ this applied in both BMI groups. Thus in overweight/obese subjects who are at high risk of developing insulin resistance, diabetes mellitus and cardiovascular diseases (Wickelgren 1998, Abbasi et al. 2002, Cervenakova et al. 2002, Grundy et al. 2004), 3SoGTT is indeed a helpful method for predicting insulin resistance.

In estimating glucose homeostasis status, 3SoGTT remains still an important method. Although insulin sensitivity indices calculated from 3SoGTT overestimate the insulin sensitivity, they can provide valuable data in identifying subjects with insulin resistance. In normal weight subjects, however, attention must be paid when choosing the appropriate approach in insulin sensitivity estimation.

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