Shape of glucose, insulin, C-peptide curves during a three hour oral glucose tolerance test: any relationship with the degree of glucose tolerance?

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Abstract

We aimed to analyze the shape of the glucose, insulin, C-peptide curves during a three hour oral glucose tolerance test (OGTT). Another aim was defining an index of shape taking into account the whole OGTT pattern. 592 OGTT curves were analyzed, mainly from women with former gestational diabetes, with glycemic concentrations characterized by normal glucose tolerance (NGT, \(n=411\)), impaired glucose metabolism (IGM, \(n=134\)) and type 2 diabetes (DM, \(n=47\)). Glucose curves were classified according to their shape (monophasic, biphasic, triphasic, 4/5-phases), and the metabolic condition of the subjects, divided according to the glucose shape stratification, was analyzed. Indices of shape based on the discrete second order derivative of the curve patterns were also defined. We found that the majority of the glucose curves were monophasic (\(n=262\)). Complex shapes were less frequent but not rare (\(n=37\) for the 4/5-phases shape, i.e., three peaks). There was a tendency towards the amelioration of the metabolic condition for increasing complexity of the shape, as indicated by lower glucose concentrations, improved insulin sensitivity and beta-cell function. The shape index computed on C-peptide, WHOSH\(_{CP}\), showed a progressive increase (monophasic: 0.93±0.04 (dimensionless); 4/5-phases: 1.35±0.14), and it showed properties typical of beta-cell function indices. We also found that the type of glucose shape is often associated to similar insulin and C-peptide shape. In conclusion, OGTT curves can be characterized by high variability, and complex OGTT shape is associated with better glucose tolerance. WHOSH\(_{CP}\) may be a powerful index of beta-cell function much simpler than model-based indices.

**Keywords:** shape index, glucose variability, pulsatile insulin secretion, insulin oscillations, beta-cell function
The 75g oral glucose tolerance test (OGTT) is currently used for the diagnosis of diabetes (2). The OGTT role for diabetes diagnosis may be scaled down for the increasing relevance of the glycated hemoglobin as possible diagnostic test (1), but the OGTT will probably remain a reference test for simple and relatively inexpensive assessment of parameters of glucose homeostasis, mainly insulin sensitivity and beta-cell function (10, 14). In fact, different tests are considered more accurate than the OGTT for the assessment of these metabolic parameters (euglycemic-hyperinsulinemic clamp for insulin sensitivity (6), and hyperglycemic clamp or intravenous glucose tolerance test for first-phase or acute insulin response (6, 8)), but these tests are complex and cumbersome for both the patients and the investigator. Thus, we expect the OGTT to maintain a relevant role in the study of glucose metabolism.

Up to now, little attention has been devoted to the possible information related to the shape of the concentration time courses of the OGTT variables. It is true that the results of some refined models of beta-cell function, such as that of the study (13), being based on the analysis of the whole OGTT data, are clearly indirectly influenced by the shape of the OGTT curve, but the analysis of the possible information related to the shape per se has never been performed in detail so far. The reference study on this issue is that of Tschritter et al. (19), but some limitations of that study prevented a complete analysis of the possible relevance of the OGTT shape. In fact, in (19) the emphasis was on the analysis of the glucose curves, whereas the insulin curves were only briefly presented and those of C-peptide totally neglected. Furthermore, the OGTT was only two hour long, which may be a strong limitation when evaluating the shape. Our aim was therefore the analysis of the shape of glucose, insulin and C-peptide curves during a three hour, frequently sampled OGTT in a large dataset including subjects which glycemic concentrations spanning from normal glucose tolerance to overt diabetes, to disclose possible information of physiological or even clinical relevance. In fact, firstly we aimed to provide a clear and complete classification of the different shapes not only of glucose, but also of insulin and C-peptide curves, with also the assessment of the prevalence of the different shape types. Also, we investigated whether there are significant
similarities in the shape of glucose, insulin and C-peptide in a single individual. Lastly, we aimed to
define a reliable shape index, and to explore its possible relationships with already known
parameters of glucose metabolism.

Materials and methods

Subjects
We analyzed 525 OGTT of women with a history of gestational diabetes, and 67 OGTT of women
with previous uncomplicated pregnancy. All women were recruited from the outpatient department
of the University Clinic of Vienna, and gave written informed consent for participation in the study,
which was approved by the local Ethics Committee. Some of the data presented here were already
included in previous studies (22, 23). The glucose curves showed values matching the ADA 2003
criteria for normal glucose tolerance (NGT, \( n=411 \)), impaired glucose metabolism (including
impaired glucose tolerance and/or impaired fasting glucose: IGM, \( n=134 \)), and type 2 diabetes
(T2DM, \( n=47 \)).

Tests
After an overnight fast, all the subjects underwent a standard 75g OGTT with venous blood
sampling at fasting and at 10, 20, 30, 60, 90, 120, 150 and 180 min after the glucose load. On a
separate day, some of the subjects (\( n=219 \)) underwent an insulin-modified intravenous glucose
tolerance test (IVGTT). Glucose (300 mg/kg) was injected in 30 seconds and insulin (0.03 IU/kg,
Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at time 20 for 5 min. Venous
blood samples for determination of plasma concentration of glucose, insulin and C-peptide were
collected at fasting and frequently for 180 min after glucose infusion.

Plasma glucose was measured with the glucose oxidase method by an automated glucose analyzer
(Beckman, Fullerton, CA), with an interassay coefficient of variation <2%. Insulin (Serono
Diagnostics, Freiburg, Germany) and C-peptide (CIS Bio International, Gif-sur-Yvette, France) were determined in duplicate by commercially available radioimmunoassay kits, with an interassay coefficient of variation <5%.

Calculation of insulin sensitivity and beta-cell function

Insulin sensitivity from OGTT was estimated through the OGIS method (12). Beta-cell function was assessed through the mathematical model of Mari et al. (13), which yields beta-cell glucose sensitivity, rate sensitivity, potentiation factor ratio, and total insulin secretion during the OGTT, TIS.

The insulin sensitivity index SI was calculated from IVGTT by minimal model analysis (3). For the beta-cell function, we considered the acute insulin response (AIR), computed as the suprabasal integral of plasma insulin in the 0-8 minute interval following the bolus, and normalized to the interval length (8). The acute C-peptide response (ACPR) was similarly computed.

Classification of shape of the OGTT curves

Glucose shape was defined “monophasic” when glucose simply showed an initial increase and subsequent decline (i.e., one peak). The shape was “biphasic” when glucose showed a further increase following the decline. The “triphasic” case was characterized by two complete peaks. These definitions were the same used in (19), but in our study the OGTT was three hour long instead of two (and more samples were present in the first hour). Thus, we were able to observe more heterogeneous and complex curves. In fact, we found some cases with “4-phases”, and also “5-phases” (i.e., three complete peaks). In the analysis, 4 and 5 phase cases were considered together. Variations in glucose values from one sample to the following sample were considered significant only when the difference was at least 2%. This criterion was necessary to avoid false detection of minima or maxima in the glucose curve. A few cases not matching the indicated
classification criteria were assumed as “unclassified”. Similar criteria were used for the classification of insulin and C-peptide curves, assuming 5% for significant variations.

*Calculation of shape indices*

We defined a new shape index based on the whole OGTT curve, i.e., all the samples of the three hour OGTT. Our index requires the calculation of the discrete second order derivative of the OGTT curve:

a) Calculation of \( \frac{\Delta G_i}{\Delta t_i}, \frac{\Delta G_2}{\Delta t_2}, \ldots, \frac{\Delta G_8}{\Delta t_8} \)

Where \( \Delta G_i \), with \( i \) from 1 to 8, is the difference between the glucose value of the sample at time \( t_{i+1} \) and that at time \( t_i \), and \( \Delta t_i \) is the corresponding time difference. For instance, for \( i=8 \), \( \Delta G_8 \) is the difference between glucose at 180 min (9th sample of the OGTT) and glucose at 150 min (8th sample), and \( \Delta t_8 \) is the corresponding time difference (180-150=30). Thus, from the 9 samples of the OGTT we obtained 8 first derivative samples.

b) Calculation of \( \frac{2^\Delta G_1}{\Delta t_1^2}, \frac{2^\Delta G_2}{\Delta t_2^2}, \ldots, \frac{2^\Delta G_7}{\Delta t_7^2} \)

With \( 2^\Delta G_i/\Delta t_i^2 = \left( \frac{\Delta G_{i+1} - \Delta G_i}{\Delta t_{i+1} - \Delta t_i} \right)^2/\Delta t_i \), \( i \) from 1 to 7. Thus, the iteration of the method described at the previous step, over each of the 8 first derivative samples, provides 7 second derivative samples.

c) The absolute value of each of the 7 second derivative samples is computed; then, the mean value among these 7 samples is considered: this is our new shape index.

d) Finally, to get a more compact and easily readable index, we normalized the value of the shape index in each subject to the mean value over all the dataset.

All the procedure above described for glucose was then repeated for insulin and C-peptide. Eventually, three shape-related indices were obtained: WHOSHGLU (WHole-Ogtt-SHape-index), WHOSHI NS, WHOSHCP, respectively.
For comparison, we also computed the Tschritter’s shape index (19), which is based on two glucose samples only (90 and 120, or 60 and 90 min).

Statistical analysis

Data are reported as mean±SE. Differences among groups were tested through ANOVA. Differences between couples of variables in a specific group were tested through paired t-test. Relationships between variables were tested with univariate and multivariate regression analysis. Nominal variables were analyzed through chi-square test. The distribution of variables was tested for normality, and in the case of non-normal distribution the statistical analyses were performed on logarithmically transformed values. P<0.05 was considered statistically significant.

Results

Parameters in the glucose shape categories

Table 1 reports the main characteristics and metabolic parameters of the subjects divided according to the type of shape of the glucose curve. The “unclassified” group was not reported, consisting only of 16 curves. Of note, mean glucose concentrations progressively decreased significantly from the monophasic to the 4/5-phases condition (only in triphasic vs. biphasic the difference did not reach statistical significance).

Prevalence of the glucose shape categories

The most common shape for glucose curve was the monophasic. Triphasic shape was also quite frequent. More complex shapes (i.e., 4/5-phases) were as expected unusual, but not rare. Biphasic shape was less frequent than the monophasic or triphasic. According to the chi-square test, the number of NGT, IGM and DM was different in the four shape groups (P<0.0001). Figure 1 shows the mean glucose curve in each group. The corresponding mean insulin and C-peptide curve in each
of the glucose shape groups are also reported. It must be noted that averaging the single patterns partially flattened down the mean pattern (this holds particularly for insulin and C-peptide curves in the 4/5-phases group). For this reason we have also reported some individual curves, which provide evidence of the oscillations. Reporting individual curves also allows underlining the heterogeneity of the OGTT patterns: for instance, as regards glucose in the monophasic group (Figure 1, panel (a)), the reported individual curve shows that the rate of glucose decrease following the peak is not necessarily uniform, as the mean curve would erroneously suggest. Another interesting individual curve was that reported for C-peptide in the biphasic group (Figure 1, panel (l)): it shows that, in some cases, after three hours from the glucose ingestion, C-peptide is not only much higher than the basal value, but it is even still increasing.

**Relationships between shape of glucose, insulin, and C-peptide curves**

Regarding the relationship among the shape of glucose and the shape of insulin or C-peptide, we divided the insulin and C-peptide curves into the same shape categories applied to glucose. For insulin, we found \( n=208 \) in monophasic, \( n=65 \) in biphasic, \( n=215 \) in triphasic, \( n=60 \) in 4/5-phases groups (\( n=44 \) unclassified); for C-peptide, we found \( n=406 \), \( n=50 \), \( n=102 \), \( n=8 \), respectively (\( n=26 \) unclassified). According to the chi-square test, the prevalence of the different shape categories was not the same among glucose, insulin and C-peptide curves (\( P<0.006 \)). However, there was a tendency to have some degree of correspondence among glucose, insulin and C-peptide shape categories: for instance, the insulin and C-peptide curves related to the monophasic glucose curves were monophasic in the 58% and 91% of cases, respectively, while they were of the 4/5-phases type in only the 7% of cases for insulin, and no cases for C-peptide. On the other hand, the highest percentage of 4/5-phases insulin and C-peptide curves were in fact found in the 4/5-phases glucose group.
Curves with continuous increase during the OGTT

It must be noted that among the insulin curves we found 8 curves with continuous increase during the OGTT period, and 41 curves with the same behavior among the C-peptide curves: we included those curves in the respective monophasic group. This suggests that in some cases not even a three hour period is sufficient to restore the basal condition of the glucose homeostasis after the administration of the 75g glucose load. This is further confirmed by the significant difference, on average over the whole population, between basal and 180 min values of both insulin and C-peptide (P<0.0001 for both), whereas for glucose the difference did not reached significance (P=0.089).

General findings related to the shape indices

\(\text{WHOSH}_{\text{GLU}}\) tended to increase from monophasic to 4/5-phases group, though the difference between the groups was not always significant. This increasing trend was of course expected: higher variability (i.e., higher number of oscillations and/or more rapid changes in the concentration curve) is likely to determine higher values of the second derivative, on which \(\text{WHOSH}_{\text{GLU}}\) is based. Similar considerations hold for \(\text{WHOSH}_{\text{INS}}\) and \(\text{WHOSH}_{\text{CP}}\). As regards \(\text{WHOSH}_{\text{INS}}\), it showed again some tendency to increase, but less clear than in \(\text{WHOSH}_{\text{GLU}}\). On the other hand, \(\text{WHOSH}_{\text{CP}}\) showed surprisingly a clear increase, even more evident than in \(\text{WHOSH}_{\text{GLU}}\) (P<0.03-P<0.0001); statistical significance was in fact not reached only between monophasic and biphasic. Differences of \(\text{WHOSH}_{\text{CP}}\) among groups remained significant even when adjusting for any of the following covariates: age, BMI, mean glucose, mean insulin, mean C-peptide.

C-peptide-based shape index: relationships with the other parameters

We further investigated the properties of the C-peptide-based index. As regards the parameters reported in Table 1, according to linear regression analysis \(\text{WHOSH}_{\text{CP}}\) showed positive relationship with basal and mean insulin and C-peptide concentrations, with glucose sensitivity, with TIS, with AIR and ACPR, as well as with \(\text{WHOSH}_{\text{GLU}}\) and \(\text{WHOSH}_{\text{INS}}\). It showed negative relationship with
age and with the glucose concentrations. Of note, \( \text{WHOSH}_{CP} \) was not related to \( S_I \), and only very slightly to OGIS. Additionally, \( \text{WHOSH}_{CP} \) was not related to BMI.

*C-peptide-based shape index: a possible surrogate of glucose sensitivity*

Since \( \text{WHOSH}_{CP} \) showed a reasonable relationship with the glucose sensitivity \((R^2=0.202, P<0.0001; \text{see Figure 2})\), additional similarities between the two parameters were investigated. \( \text{WHOSH}_{CP} \) was negatively related to the mean glucose during the OGTT, though the relation was very weak \((R^2=0.053, P<0.0001; \text{Figure 2})\). Similar behavior was observed for glucose sensitivity, with stronger relationship \((R^2=0.304, P<0.0001)\). Furthermore, \( \text{WHOSH}_{CP} \) was positively related to the parameters of beta-cell function derived from the IVGTT \((R^2=0.221 \text{ for AIR, } R^2=0.155 \text{ for ACPR, } P<0.0001; \text{Figure 2})\), and again similar results were found for glucose sensitivity \((R^2=0.363 \text{ for AIR, } R^2=0.408 \text{ for ACPR, } P<0.0001)\). At contrast, \( \text{WHOSH}_{CP} \) was not related with \( S_I \) \((P=0.7)\) and very marginally with OGIS \((R^2=0.011, P=0.02)\), and essentially similar behavior was observed for glucose sensitivity for both \( S_I \) \((P=0.3)\) and OGIS \((R^2=0.045, P<0.0001)\).

As regards differences among groups, \( \text{WHOSH}_{CP} \) was different among NGT, IGM, DM \( (1.07\pm0.03, 0.93\pm0.05, 0.67\pm0.05, \text{ respectively, } P<0.004)\), and similar differences were observed as expected with glucose sensitivity \((116.0\pm2.8, 74.7\pm2.9, 37.3\pm2.7 \text{ pmol min}^{-1} \text{ m}^{-2} \text{ mM}^{-1}, P<0.0001)\).

Through multivariate regression analysis, we looked for the major determinants of mean glucose during the OGTT. Significant determinants were age, BMI, OGIS, glucose sensitivity \((R^2=0.741, P<0.02 \text{ for all the covariates})\). When \( \text{WHOSH}_{CP} \) was added to the analysis, it did not result a significant covariate. However, if glucose sensitivity was not included within the covariates, the role of \( \text{WHOSH}_{CP} \) became significant and similar results were found \((R^2=0.658, P<0.001 \text{ for all the covariates})\). In contrast, when any of the other covariates (age, BMI, OGIS) was excluded, in the presence of glucose sensitivity \( \text{WHOSH}_{CP} \) again resulted not significant. Based on the reported findings, we can conclude that \( \text{WHOSH}_{CP} \) can be a surrogate of the glucose sensitivity parameter.
**Beta-cell function indices in normotolerant subjects**

To further investigate the properties of WHOSH\textsubscript{CP} as a beta-cell function index, we divided the NGT group into two subgroups: those with former gestational diabetes (fGDM, \(n=344\)) and those with previous normal pregnancy (CNT, \(n=67\)). We found that WHOSH\textsubscript{CP} was different in the two groups (fGDM: 1.02±0.03, CNT: 1.30±0.11, \(P=0.0093\)), and similarly for glucose sensitivity (113.8±3.0 vs. 135.5±8.5 pmol min\(^{-1}\) m\(^{-2}\) mM\(^{-1}\), \(P=0.0089\)), which was already proved to be slightly different in normotolerant fGDM women compared to control subjects (22).

The presence of a slight beta-cell function defect, disclosed by WHOSH\textsubscript{CP}, was further confirmed by IVGTT analysis. In the subgroup of NGT where IVGTT data were available (\(n=121\) for fGDM, \(n=43\) for CNT), both AIR and ACPR showed a tendency toward a slight beta-cell function impairment in fGDM, though the difference did not reach statistical significance (216.4±14.0 vs. 289.0±33.5 pmol/l, \(P=0.07\) for AIR; 761.7±30.9 vs. 885.0±61.0 pmol/l, \(P=0.06\) for ACPR).

However, when we considered the product \(S_1\times\text{AIR}\), assumed by some investigators as a further index of beta-cell function (the so-called “disposition index”), it showed in fact a significant difference, and similarly for \(S_1\times\text{ACPR}\) (\(P=0.0025\) and \(P=0.0057\), respectively), in agreement with WHOSH\textsubscript{CP}. Of note, as regards other empirical indices from the OGTT such as the insulinogenic index, the mean insulinaemia normalized to the mean glycaemia over the OGTT, or corresponding indices based on C-peptide, none of them was able to disclose differences between fGDM and CNT (not shown).

Further analysis was performed by dividing the NGT subjects according to their belonging to the different glucose shape groups: those characterized by monophasic shape, NGT\textsubscript{MONO} (\(n=138\)), and those in the other shape groups, NGT\textsubscript{NON-MONO} (\(n=266\)). We found that glucose sensitivity was different, though slightly, between the two groups (NGT\textsubscript{MONO}: 111.3±5.0, NGT\textsubscript{NON-MONO}: 123.5±4.0 pmol min\(^{-1}\) m\(^{-2}\) mM\(^{-1}\), \(P=0.0375\)), whereas WHOSH\textsubscript{CP} tended to be lower in NGT\textsubscript{MONO}, but the difference did not reached statistical significance (1.04±0.06 vs. 1.13±0.05, \(P=0.0853\)). As regards the insulinogenic index, and AIR, ACPR (where available), none of them was significantly
different in the two groups (P>0.1). Only with the product $S_1 \times ACPR$, a slightly lower value was found in NGT_{MONO} (P=0.03).

Comparison with results of previous studies

According to the classification criteria for glucose shape of the study (19), our 592 OGTT glucose curves (with reduced sampling, i.e., only two hours, with the samples present in that study) showed monophasic shape in 328 cases and biphasic shape in 147 cases (including a few triphasic cases that in (19) were included in the biphasic group). The unclassified type was found in 117 cases. These results were in relatively fair agreement with those of the study (19), where the monophasic shape was prevailing over the biphasic shape, and with a relatively high number of unclassified cases. As regards the Tschritter’s shape index, in our case it was equal to $-1.56\pm0.06$ mmol/l in the monophasic group and $0.85\pm0.04$ mmol/l in the biphasic group; in the whole population it showed a negative relationship with the mean glucose concentration of the OGTT ($R^2=0.19$, $P<0.0001$), in agreement with (19).

Discussion

In this study we analyzed the shape of glucose, insulin and C-peptide OGTT curves. The novelties of our study were: i) analysis of three hour OGTT period, with frequent sampling in the first hour; ii) inclusion of glucose data varying in a wide range, spanning from normotolerance to overt diabetes; iii) deep shape analysis not only of glucose but also of insulin and C-peptide curves; iv) definition of shape indices based on the whole OGTT data. Thus, our study represents a significant improvement compared to the first study on the OGTT shape (19): in that study, only two hour, not frequently sampled glucose data were considered (insulin was presented marginally and C-peptide not even reported), diabetic subjects were lacking, and the shape index was based on two glucose
samples only. Another study was found in the literature based on OGTT shape (9), but it essentially shared the limitations of the study (19).

Our results in part agree with what reported in (19): first, the fact that for glucose the simple monophasic (one peak) shape is the most common shape; second, the finding that more complex shapes (biphasic vs. monophasic in (19)) are associated with better glucose tolerance (i.e., lower glucose concentrations). However, probably due to the longer OGTT period, we were able to observe also a large number of glucose curves with triphasic shape, and even with 4/5-phases (three peaks), which were obviously associated to increasing values of the glucose-based shape index, WHOSHGLU (that, however, must not be considered at all as an alternative way to define the glucose tolerance of one subject). Such more complex shapes were observed also in the insulin and C-peptide curves, though for C-peptide the more complex shapes were rare. This may be due to the fact that C-peptide has lower plasma clearance than insulin (21), and hence three hours may be not sufficient to develop the whole pattern of plasma concentration. Another observation is that the biphasic shape is much less frequent than the monophasic or triphasic. It may be due to the reason that actual biphasic shape, according to its definition, would require that at least one OGTT sample shows a value under the basal, and this condition may be not very common. Thus, it is possible that with a longer observation period some biphasic curves would in fact be found triphasic, and hence the number of biphasic cases would be even smaller.

The number of cases in each shape group may be influenced by the minimum difference between samples that we assumed to be significant. For insulin and C-peptide we assumed 5% difference, whereas for glucose we assumed 2% only, as is the interassay coefficient of variation for glucose measurements. However, we also analyzed the effect of assuming for glucose the same 5% criterion used for insulin and C-peptide, repeating all the main analyses of our study in this condition. As expected, there was a tendency of some glucose curves to migrate towards the shape groups at lower complexity (monophasic: n=309; biphasic: n=76; triphasic: n=165; 4/5-phases: n=26). However, none of the relevant findings and conclusions of our study was significantly affected (not
shown). Thus, we confidently affirm that the value assumed for the detection of significant glucose changes was not critical, at least in the 2%-5% interval.

The main result with physiological implications is that increasing variability of the OGTT (i.e., categories at higher phases) corresponds to an improvement of the metabolic condition, indicated by the lower glucose concentrations. The groups with lower variability (monophasic and biphasic) tend to compensate their higher glucose concentrations with higher insulin secretion (see TIS, as well as mean insulin and C-peptide concentrations). However, from the glucose concentrations it appears that such compensation is not sufficient, and this is mirrored by the worse beta-cell function observed in these groups (see in particular the glucose sensitivity). Furthermore, also insulin sensitivity tends to be lower. As a matter of fact, almost all the diabetic cases ($n=36$ over 43) are included in the monophasic group, whereas the majority of the normotolerant cases ($n=206$ over 404) are included in the triphasic and 4/5 phases groups (see Table 1).

What is the reason of these findings, and what regulatory mechanisms are possibly involved? The study (19) seems to conclude that the better metabolic condition observed in the biphasic compared to the monophasic group is probably only marginally a matter of shape, but it is due to the lower glycemic concentrations. This observation, however, does not explain why there is in fact a significant relationship between lower glucose concentrations (with better insulin sensitivity and beta-cell function) and higher variability in the OGTT curves of glucose (but also of insulin and C-peptide). Many studies have shown that insulin secretion (and consequently plasma insulin concentration) is characterized by some degree of pulsatility (15). Insulin pulsatility includes rapid oscillations (period of 5-15 min), but also ultradian oscillations with period of 80-150 min (16), and even circadian oscillations (24). Some studies also showed that insulin oscillations can be coupled to glucose oscillations of similar period (16, 17), and it was suggested that the oscillatory behavior may be an intrinsic characteristic of the insulin-glucose regulation system in healthy individuals. Some studies found in fact that these oscillations are depressed in diabetic (18) or elderly (16)
individuals, and it was claimed that this may be sign of a specific defect in beta-cell function, i.e. a reduced dynamic responsiveness of insulin secretion to glucose changes.

The studies (15-18, 24) did not report results during an OGTT. To the best of our knowledge, our study is the first one that reports observations on insulin and C-peptide oscillations during an OGTT. Nonetheless, our results appear consistent with those of the previous studies, such as (18); indeed, we found higher variability in the healthier individuals. Also, the hypothesis that reduced pulsatility may be due to a beta-cell function defect (16) is compatible with our findings on the C-peptide-based shape index, WHOSHCP, which was found to replicate the behavior of the glucose sensitivity, that is in fact a beta-cell function parameter of proven relevance and reliability (11). Furthermore, also the observation that insulin oscillations can be coupled to similar glucose oscillations is in agreement with our results, since we found that higher variability in the glucose curve tends to be reflected by higher variability in insulin (and partially C-peptide) curves.

What may cause the oscillations during the OGTT remain unclear in our study. As regards the glucose oscillations, given the fact that during an OGTT the endogenous glucose production is likely to be almost suppressed, it can be suggested that in such conditions oscillations in plasma glucose may be entrained by oscillations in glucose absorption (4). However, this cannot be demonstrated by our data, since we did not measure glucose absorption, and this must be acknowledged as a limitation of our study.

It is known that the pathogenesis of type 2 diabetes is related to three main physiologic defects: insulin resistance in muscle and liver, and impairment in beta-cell function. Recently, it has become evident that beta-cell function plays a role more important than what previously expected: it appears that it is the progressive impairment in beta-cell function that actually determines the rate of progression towards type 2 diabetes (5). In some recent studies, such as (7), it was shown that the OGTT may be not completely adequate for the assessment of insulin resistance/sensitivity indices. Instead, there is increasing evidence that the OGTT should be adequate for reliable estimation of beta-cell function, if appropriate methods are used (11). These considerations suggest that OGTT
analysis for the assessment of beta-cell function is relevant and appropriate. In this study, we found that our shape index based on the C-peptide OGTT data, WHOSH\textsubscript{CP}, may in fact have clinical relevance in the assessment of beta-cell function. It is able to disclose differences in beta-cell function not only among groups with different glucose tolerance (NGT, IGM, DM), but also between groups with glucose values all in the normal range (see normotolerant fGDM and CNT). Furthermore, WHOSH\textsubscript{CP} requires only C-peptide data, and it is easy to be computed (in condition of discrete, not continuous data, derivatives reduce simply to differences). Thus, differently to the glucose sensitivity (13), it does not need sophisticated modeling analysis: a simple spreadsheet is adequate to implement the index formula, and hence there is no need of more complex mathematical tools. On the other hand, despite similar simplicity, WHOSH\textsubscript{CP} should be more reliable than other empirical beta-cell function parameters from the OGTT, such as the insulinogenic index, which is hardly capable of discriminating between subtle differences in beta-cell function, and is known to be prone to outliers and even negative values (20), as it occurred also in the dataset of this study (not shown). A simplified version of WHOSH\textsubscript{CP} was also computed, based only on the samples of the traditional two hour OGTT (i.e., 0, 30, 60, 90, 120 min). Surprisingly, many properties of the index, both in terms of difference between groups and in terms of similarities with glucose sensitivity, were confirmed (not shown), indicating that the use of a short OGTT is likely to have effects on the shape-based classification of the OGTT curves, but not on the main properties of our shape index. We conclude that WHOSH\textsubscript{CP} may be applied in the clinical contest for simple estimation of beta-cell function, when for any reason sophisticated modeling analysis cannot be done.

We have already reported some limitations of our study. Another limitation relies in the type of dataset. In fact, the analyzed dataset was very rich (three hour frequently sampled complete OGTT, and IVGTT in a wide subgroup), but it mainly consisted of a specific population, i.e., women with a history of gestational diabetes. Thus, it cannot be completely excluded that some of our results may be somewhat different in another population. On the other hand, it must be noted that our dataset
also included some women without former gestational diabetes. Furthermore, previous studies indicate that in women with former gestational diabetes (normotolerant after delivery), when compared to healthy control women with similar age, BMI and glucose concentrations, only slight differences can be identified in the main parameters of glucose metabolism (23). Thus, we did not expect that the results presented in this study are strongly affected by the presence of former gestational diabetes. Another issue is the fact that the dataset included only women, thus without the possibility of drawing conclusions about possible gender-related differences. However, we subsequently analyzed a small dataset of two hour OGTT data including both females ($n=49$) and males ($n=23$) with glucose tolerance spanning from normoglycemia to overt diabetes (not reported in Results section): females and males, which on average had similar age, BMI, and glycemic concentrations, showed no significant difference in any of the shape indices ($P=0.09–P=0.4$), thus suggesting that gender differences should not be relevant in the analysis of the OGTT shape. It should also be noted that the number of diabetic patients in our dataset was small (around 10% only). However, it was found that the large majority of them were characterized by monophasic glucose shape (see Table 1). Thus, the possible presence of further diabetic subjects would probably mainly reflect in other monophasic cases, but this shape group was already well represented in our study ($n=262$), and hence adding some more cases would probably not change the main findings and conclusions of the study.

**Perspectives and significance**

Given the interest on the clinical use of the OGTT, we studied in detail what information can be provided by the analysis of the curves shape. Indeed, our study represents a significant step forward in the analysis of the OGTT shape and its relationship with the glucose tolerance condition. The main findings and conclusions are: *i*) OGTT curves can be characterized by high variability, i.e., complex shape; *ii*) the type of shape of the glucose curve tends to be mirrored by similar shape in the insulin and C-peptide curve; *iii*) complex shape is associated with better glucose tolerance
condition; iv) a new shape index based on C-peptide may be viewed as a simple empirical index of beta-cell function that can be easily used in clinical settings.

Acknowledgements

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Disclosures

The authors have no conflict-of-interest relevant to this article to declare.
References


Legends for figures

Figure 1 - Glucose curve during the OGTT (mean±SE) in the monophasic (a), biphasic (b), triphasic (c), 4/5-phases groups (d); corresponding insulin (e-h) and C-peptide (i-n) curves are also reported (solid lines). An individual profile belonging to each group of curves is also reported (dashed lines).

Figure 2 – Relationship between WHOSHCP and glucose sensitivity (a), mean glucose (b), AIR (c), and ACPR (d). In the inserts, the relationship between glucose sensitivity and the same variables are reported. All the data are logarithmically transformed. Different symbols are used for the different glucose shape groups (circle: monophasic; square: biphasic; triangle: triphasic; rhomb: 4/5- phases).
Tables. Table 1 – Main characteristics and metabolic parameters of the subjects classified according to the shape of the OGTT glucose curve (mean±SE). Parameters are derived from the OGTT unless otherwise specified.

<table>
<thead>
<tr>
<th></th>
<th>Monophasic</th>
<th>Biphasic</th>
<th>Triphasic</th>
<th>4/5-Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>262</td>
<td>76</td>
<td>201</td>
<td>37</td>
</tr>
<tr>
<td>NGT/IGM/DM</td>
<td>138/88/36</td>
<td>60/13/3</td>
<td>170/27/4</td>
<td>36/1/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.5±0.3</td>
<td>35.4±0.7</td>
<td>34.7±0.4</td>
<td>32.4±0.9</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.3±0.4</td>
<td>25.3±0.7</td>
<td>25.6±0.4</td>
<td>24.6±0.8</td>
</tr>
<tr>
<td>Basal Glucose (mmol/l)</td>
<td>5.43±0.07</td>
<td>5.06±0.09</td>
<td>4.93±0.04</td>
<td>4.69±0.05</td>
</tr>
<tr>
<td>Glucose at 120min (mmol/l)</td>
<td>7.99±0.20</td>
<td>5.90±0.22</td>
<td>6.08±0.12</td>
<td>5.37±0.16</td>
</tr>
<tr>
<td>Glucose at 180min (mmol/l)</td>
<td>5.66±0.18</td>
<td>6.02±0.24</td>
<td>4.73±0.10</td>
<td>4.97±0.17</td>
</tr>
<tr>
<td>Mean Glucose (mmol/l)</td>
<td>8.25±0.16</td>
<td>6.75±0.18</td>
<td>6.35±0.10</td>
<td>5.57±0.14</td>
</tr>
<tr>
<td>Basal Insulin (pmol/l)</td>
<td>79.0±2.9</td>
<td>65.8±3.9</td>
<td>61.1±3.2</td>
<td>60.3±5.6</td>
</tr>
<tr>
<td>Insulin at 180min (pmol/l)</td>
<td>274.4±16.3</td>
<td>280.7±27.8</td>
<td>169.7±11.9</td>
<td>191.3±24.5</td>
</tr>
<tr>
<td>Mean Insulin (pmol/l)</td>
<td>422.1±13.5</td>
<td>315.3±21.6</td>
<td>302.7±13.6</td>
<td>282.9±25.9</td>
</tr>
<tr>
<td>Basal C-peptide (pmol/l)</td>
<td>752.6±22.2</td>
<td>639.6±35.2</td>
<td>627.0±18.4</td>
<td>590.4±44.9</td>
</tr>
<tr>
<td>C-peptide at 180min (pmol/l)</td>
<td>2273±70</td>
<td>2096±131</td>
<td>1792±57</td>
<td>1840±136</td>
</tr>
<tr>
<td>Mean C-peptide (pmol/l)</td>
<td>2575±53</td>
<td>2155±93</td>
<td>2194±49</td>
<td>2064±123</td>
</tr>
</tbody>
</table>

**Beta-cell function**

<table>
<thead>
<tr>
<th></th>
<th>Monophasic</th>
<th>Biphasic</th>
<th>Triphasic</th>
<th>4/5-Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Sensitivity (pmol min⁻¹ m⁻² mM⁻¹)</td>
<td>86.9±3.4</td>
<td>93.5±4.8</td>
<td>115.6±4.0</td>
<td>134.6±8.6</td>
</tr>
<tr>
<td>Rate Sensitivity (pmol m⁻² mM⁻¹)</td>
<td>571±30</td>
<td>555±68</td>
<td>736±37</td>
<td>808±97</td>
</tr>
<tr>
<td>Potentiation factor ratio (dimensionless)</td>
<td>1.61±0.06</td>
<td>1.51±0.04</td>
<td>1.40±0.03</td>
<td>1.53±0.08</td>
</tr>
<tr>
<td>TIS (nmol m⁻²)</td>
<td>69.2±1.4</td>
<td>59.9±2.8</td>
<td>59.4±1.4</td>
<td>56.7±3.4</td>
</tr>
<tr>
<td>AIR⁺ (pmol/l)</td>
<td>188.3±15.3</td>
<td>169.5±19.4</td>
<td>234.8±22.0</td>
<td>314.8±53.0</td>
</tr>
<tr>
<td>ACPR⁺ (pmol/l)</td>
<td>630.8±38.0</td>
<td>613.2±49.7</td>
<td>596.4±46.5</td>
<td>962.7±97.9</td>
</tr>
</tbody>
</table>

**Insulin sensitivity**

<table>
<thead>
<tr>
<th></th>
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<th>Biphasic</th>
<th>Triphasic</th>
<th>4/5-Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGIS (ml min⁻¹ m⁻²)</td>
<td>395.2±6.0</td>
<td>398.0±10.7</td>
<td>454.8±5.6</td>
<td>452.0±11.4</td>
</tr>
<tr>
<td>S¹ (10⁻⁵ min⁻¹ (μU/ml)⁻¹)</td>
<td>3.52±0.24</td>
<td>5.92±0.65</td>
<td>4.96±0.37</td>
<td>5.10±0.72</td>
</tr>
</tbody>
</table>

**Whole OGTT-based shape indices**

<table>
<thead>
<tr>
<th></th>
<th>Monophasic</th>
<th>Biphasic</th>
<th>Triphasic</th>
<th>4/5-Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHOSHGLU (dimensionless)</td>
<td>0.95±0.02</td>
<td>0.96±0.04</td>
<td>1.02±0.03</td>
<td>1.13±0.08</td>
</tr>
<tr>
<td>WHOSHINS (dimensionless)</td>
<td>0.95±0.04</td>
<td>0.87±0.07</td>
<td>1.05±0.06</td>
<td>1.36±0.20</td>
</tr>
<tr>
<td>WHOSHCP (dimensionless)</td>
<td>0.93±0.04</td>
<td>0.88±0.05</td>
<td>1.09±0.04</td>
<td>1.35±0.14</td>
</tr>
</tbody>
</table>

¹ Significant difference between Biphasic and Monophasic; ² Triphasic and Monophasic; ³ Triphasic and Biphasic; ⁴ 4/5-Phases and Monophasic; ⁵ 4/5-Phases and Biphasic; ⁶ 4/5-Phases and Triphasic. * From IVGTT (n=219).
Mean glucose (mmol/l)

\[ y = 0.51 x + 4.52 \]

\[ y = 0.11 x + 1.93 \]

Mean glucose sensitivity (pmol min\(^{-1}\) m\(^{-2}\) mM\(^{-1}\))

\[ y = -0.24 x + 3.01 \]

\[ y = -0.11 x + 1.93 \]

ACPR (pmol/l)

\[ y = 0.87 x + 2.48 \]

\[ y = 0.74 x + 5.17 \]

AIR (pmol/l)

\[ y = 0.91 x + 1.03 \]

\[ y = 0.56 x + 6.42 \]