Shape of glucose, insulin, C-peptide curves during a 3-h oral glucose tolerance test: any relationship with the degree of glucose tolerance?

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The 75-g oral glucose tolerance test (OGTT) is currently used for the diagnosis of diabetes (6a). The OGTT role for diabetes diagnosis may be scaled down for the increasing relevance of the glycated hemoglobin as a possible diagnostic test (7a), but the OGTT will probably remain a reference test for simple and relatively inexpensive assessment of parameters of glucose homeostasis, mainly insulin sensitivity and β-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 β-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 this article (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 2 h 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 3-h, frequently sampled OGTT in a large data set, including subjects that glycemic concentrations spanning from normal glucose tolerance to overt diabetes, to disclose possible information of physiological or even clinical relevance. In fact, first, 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. All participants 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 American Diabetes Association 2003 criteria for normal glucose tolerance (NGT, n = 411), impaired glucose metabolism (IGM; including impaired glucose tolerance and/or impaired fasting glucose: IGM, n = 134), and Type 2 diabetes (n = 47).

Tests. After an overnight fast, all the subjects underwent a standard 75-g 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 \((300 \text{ mg/kg})\) was injected in 30 s, and insulin \(0.03 \text{ UI/kg}; \text{Humulin R; Eli Lilly, Indianapolis, IN}\) 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 oral glucose insulin sensitivity (OGIS) method \((12)\). Beta-cell function was assessed through the mathematical model of Mari et al. \((13)\), which yields \(\beta\)-cell function, \(\beta\)-cell function.

Insulin sensitivity index \(S_i\) was calculated from IVGTT by minimal model analysis \((1)\). For the \(\beta\)-cell function, we considered the acute insulin response (AIR), computed as the suprabasal integral of plasma insulin in the 0–8-min 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 3 h long instead of 2 (and more samples were present in the 1st h). 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 \((4/5\text{-phases})\) 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 of the samples of the 3 h OGTT.

Our index requires the calculation of the discrete second-order derivative of the OGTT curve: 1) calculation of \(\Delta G_i/\Delta t_i, \Delta G_i/\Delta t_2, \ldots, \Delta G_i/\Delta t_n, \) where \(\Delta G_i\), with \(i\) from 1 to 8, is the difference between the glucose value of the sample at time \(t_i\), and that at time \(t_{i+1}\), 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 \((9th\text{ sample of the OGTT})\) and glucose at 150 min \((8th\text{ sample})\), and \(\Delta t_8\) is the corresponding time difference \((180 – 150 = 30)\). Thus, from the nine samples of the OGTT, we obtained eight first-derivative samples. 2) Calculation of \(\Delta^2 G_i/\Delta t_j^2, \Delta^2 G_i/\Delta t_j^2, \ldots, \Delta^2 G_i/\Delta t_j^2, \) with \(\Delta^2 G_i/\Delta t_j^2 = (\Delta G_{i+1} – \Delta G_i)/\Delta t_{j+1} – (\Delta G_i – \Delta G_{i-1})/\Delta t_j, \) from 1 to 7. Thus, the iteration of the method described at the previous step, over each of the eight first-derivative samples, provides seven second-derivative samples. 3) The absolute value of each of the seven second-derivative samples is computed; then, the mean value among these seven samples is considered: this is our new shape index. 4) 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 data set.

The entire procedure for glucose described above was then repeated for insulin and C-peptide. Eventually, three shape-related indices were obtained: WHOSH\text{GLU} (Whole-Oggtt-Shape-index–glucose), WHOSH–insulin (WHOSH\text{INS}), and WHOSH\text{CP}, respectively.

For comparison, we also computed the Tschirrè’s shape index \((19)\), which is based on two glucose samples only \((90\text{ and }120\text{, or }60\text{ and }90\text{ min}).

**Statistical analysis.** Data are reported as means ± 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 \(\chi^2\)-test. The distribution of variables was tested for normality, and in the case of nonnormal 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 \((only in\text{ triphasic vs. biphasic, the difference did not reach statistical significance})\).

**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 \((i.e., 4/5\text{-phases})\) were as expected unusual, but not rare. Biphasic shape was less frequent than the monophasic or triphasic. According to the \(\chi^2\)-test, the number of NGT, IGM, and diabetes mellitus \((DM)\) 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 \((this\text{ holds particularly for insulin and C-peptide curves in the 4/5-phases group})\). 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, regarding glucose in the monophasic group \((Fig. 1A)\), 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 \((Fig. 1J)\): it shows that, in some cases, after 3 h 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\text{ unclassified}); for C-peptide, we found \(n = 406, n = 50, n = 102, n = 8\), respectively \((n = 26\text{ unclassified})\). According to the \(\chi^2\)-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
Table 1. Main characteristics and metabolic parameters of the subjects classified according to the shape of the OGTT glucose curve

<table>
<thead>
<tr>
<th>Parameter</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>36/10</td>
</tr>
<tr>
<td>Age, yr</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 120 min, 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 180 min, mmol/l</td>
<td>5.68 ± 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>8.23 ± 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>79.3 ± 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 180 min, 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 180 min, pmol/l</td>
<td>227.3 ± 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 ± 93c</td>
<td>2194 ± 49c</td>
<td>206 ± 124</td>
</tr>
</tbody>
</table>

β-cell function

| Glucose sensitivity, pmol·min⁻¹·m⁻²·mM⁻¹ | 86.9 ± 3.4 | 93.5 ± 4.8 | 115.6 ± 4.0 | 134.6 ± 8.6 |
| Rate of sensitivity, pmol·m⁻²·mM⁻¹     | 571 ± 30   | 555 ± 68   | 736 ± 37    | 808 ± 97    |
| Potentiation factor ratio (dimensionless) | 1.61 ± 0.06 | 1.51 ± 0.04 | 1.40 ± 0.03 | 1.53 ± 0.08 |
| TIS, nmol/m²                          | 69.2 ± 1.4 | 59.9 ± 2.8 | 59.4 ± 1.4  | 56.7 ± 3.4  |
| AIR, pmol/l                           | 188.3 ± 15.3 | 169.5 ± 19.4 | 234.8 ± 22.0 | 314.8 ± 53.0 |
| ACPR, pmol/l                          | 630.8 ± 38.0 | 613.2 ± 49.7 | 596.4 ± 46.5 | 962.7 ± 97.9 |
| Insulin sensitivity                   | 395 ± 6.0 | 398.9 ± 10.7 | 454.8 ± 5.6 | 452.0 ± 11.4 |
| OGIS, ml·min⁻¹·m⁻²                    | 3.52 ± 0.24 | 5.92 ± 0.65 | 4.96 ± 0.37 | 5.10 ± 0.72 |
| S₀, 10⁻⁴·min⁻¹ (µU/ml)⁻¹ · ε         | 0.95 ± 0.02 | 0.96 ± 0.04 | 1.02 ± 0.03 | 1.13 ± 0.08 |
| WHOSHGLU (dimensionless)             | 0.95 ± 0.04 | 0.87 ± 0.07 | 1.05 ± 0.06 | 1.36 ± 0.20 |
| WHOSHCNS (dimensionless)             | 0.93 ± 0.04 | 0.88 ± 0.05 | 1.09 ± 0.04 | 1.35 ± 0.14 |
| WHOSHCP (dimensionless)              | 0.93 ± 0.04 | 0.88 ± 0.05 | 1.09 ± 0.04 | 1.35 ± 0.14 |
| WHOSHGLU                             | 0.95 ± 0.04 | 0.87 ± 0.07 | 1.05 ± 0.06 | 1.36 ± 0.20 |
| WHOSHCNS                             | 0.93 ± 0.04 | 0.88 ± 0.05 | 1.09 ± 0.04 | 1.35 ± 0.14 |
| WHOSHCP                              | 0.93 ± 0.04 | 0.88 ± 0.05 | 1.09 ± 0.04 | 1.35 ± 0.14 |

Values are expressed as means ± SE. OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; IGM, impaired glucose metabolism; DM, type 2 diabetes; BMI, body mass index; TIS, total insulin secretion; AIR, acute insulin response; ACPR, acute C-peptide response; OGIS, oral glucose insulin sensitivity; SI, insulin sensitivity index; WHOSH, Whole-OgTT-Shape-index; GLU, glucose; INS, insulin; CP, C-peptide. Parameters are derived from the OGTT unless otherwise specified. * Significant difference between biphasic and monophasic; † significant difference between triphasic and monophasic; ‡ significant difference between biphasic and triphasic; § significant difference between 4/5-phases and monophasic; ¶ significant difference 4/5-phases and biphasic; ‡ significant difference between 4/5-phases and triphasic; and † from intravenous glucose tolerance test (n = 219).

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 3-h period is sufficient to restore the basal condition of the glucose homeostasis after the administration of the 75-g 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 reach significance (P = 0.089).

General findings related to the shape indices. WHOSHGLU 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 WHOSHGLU is based. Similar considerations hold for WHOSHCNS and WHOSHCP. Regarding WHOSHCP, it showed again some tendency to increase, but less clear than in WHOSHGLU. On the other hand, WHOSHCP showed surprisingly a clear increase, even more evident than in WHOSHGLU (P < 0.03 – P < 0.0001); statistical significance was, in fact, not reached only between monophasic and biphasic. Differences of WHOSHCP among groups remained significant, even when adjusting for any of the following covariates: age, BMI, mean glucose, mean insulin, and mean C-peptide.

C-peptide-based shape index: relationships with the other parameters. We further investigated the properties of the C-peptide-based index. Regarding the parameters reported in Table 1, according to linear regression analysis, WHOSHCP showed a positive relationship with basal and mean insulin and C-peptide concentrations, glucose sensitivity, TIS, AIR, and ACPR, as well as WHOSHGLU and WHOSHCNS. It showed a negative relationship with age and with the glucose concentrations. Of note, WHOSHCP was not related to S₀ and only very slightly to OGIS. Additionally, WHOSHCP was not related to BMI.

C-peptide-based shape index: a possible surrogate of glucose sensitivity. Since WHOSHCP showed a reasonable relationship with the glucose sensitivity (R² = 0.202, P < 0.0001; see Fig. 2), additional similarities between the two parameters were investigated. WHOSHCP was negatively related to the mean glucose during the OGTT, although the relation was very weak (R² = 0.053, P < 0.0001; Fig. 2). Similar behavior was observed for glucose sensitivity, with a stronger relationship.
\( R^2 = 0.304, P < 0.0001 \). Furthermore, WHOSH\textsubscript{CP} was positively related to the parameters of \( \beta \)-cell function derived from the IVGTT (\( R^2 = 0.221 \) for AIR, \( R^2 = 0.155 \) for ACPR, \( P < 0.0001 \); Fig. 2), and again similar results were found for glucose sensitivity (\( R^2 = 0.363 \) for AIR, \( R^2 = 0.408 \) for ACPR, \( P < 0.0001 \)). At contrast, WHOSH\textsubscript{CP} was not related with SI (\( P = 0.7 \) and very marginally with OGIS (\( R^2 = 0.011, P = 0.02 \)), and essentially similar behavior was observed for glucose sensitivity for both SI (\( P = 0.3 \)) and OGIS (\( R^2 = 0.045, P < 0.0001 \)).

Regarding differences among groups, WHOSH\textsubscript{CP} was different among NGT, IGM, DM (1.07 ± 0.03, 0.93 ± 0.05, 0.67 ± 0.05, respectively, \( P < 0.0004 \)), and similar differences were observed as expected with glucose sensitivity (116.0 ± 2.8, 74.7 ± 2.9, 37.3 ± 2.7 pmol·min\(^{-1}\)·m\(^{-2}\)·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, and glucose sensitivity (\( R^2 = 0.741, P < 0.02 \) for all the covariates). When WHOSH\textsubscript{CP} was added to the analysis, it did not produce a significant covariate. However, when glucose sensitivity was not included within the covariates, the role of WHOSH\textsubscript{CP} became significant, and similar results were found (\( R^2 = 0.658, P < 0.0001 \) for all the covariates). In contrast, when any of the other covariates (age, BMI, OGIS) was excluded, in the presence of glucose sensitivity, WHOSH\textsubscript{CP} again produced non-significant results. On the basis of the reported findings, we can conclude that WHOSH\textsubscript{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 [control (CNT), \( n = 67 \)]. We

Fig. 1. Glucose curve during the OGTT (means ± SE) in the monophasic (A), biphasic (B), triphasic (C), 4/5-phase groups (D); corresponding insulin (E–H) and C-peptide (I–L) curves are also reported (solid lines). An individual profile belonging to each group of curves is also reported (dashed lines).
found that WHOSHCP was different in the two groups (fGDM: 1.02 ± 0.03, CNT: 1.30 ± 0.11, \( P = 0.0093 \)), and similar for glucose sensitivity (113.8 ± 3.0 vs. 135.5 ± 8.5 pmol min\(^{-1}\)m\(^{-2}\)M\(^{-1}\), \( P = 0.0089 \)), which had already proved to be slightly different in normotolerant fGDM women compared with control subjects (22).

The presence of a slight \( \beta \)-cell function defect, disclosed by WHOSHCP, 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, although 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 \( S_I \times \text{AIR} \), 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 \( S_I \times \text{ACPR} \) (\( P = 0.0025 \) and \( P = 0.0057 \), respectively), in agreement with WHOSHCP. Of note, of the other empirical indices from the OGTT, such as the insulinogenic index, the AIR, nor the ACPR (where available) was significantly different in the two groups (\( P > 0.1 \)). Only with the product \( S_I \times \text{ACPR} \) was a slightly lower value found in NGTMONO (\( P = 0.03 \)).

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 2 h, 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 Tschritter et al. (19) included in the biphasic group]. The unclassified type was found in 117 cases. Our results were in relatively fair agreement with those of Tschritter et al. (19), in which the monophasic shape was prevailing over the biphasic shape and there were a relatively high number of unclassified cases. In our study, the Tschritter’s shape index was equal to −1.56 ± 0.06 mmol/l in the monophasic group and 0.85 ± 0.04 mmol/l in the
biphasic group; in the whole population, our results showed a negative relationship with the mean glucose concentration of the OGTT ($R^2 = 0.19$, $P < 0.0001$), in agreement with Tschritter et al. (19).

**DISCUSSION**

In this study, we analyzed the shape of glucose, insulin, and C-peptide OGTT curves. The novel features of our study were 

1) an analysis of a 3-h OGTT period, with frequent sampling in the first hour; 2) an inclusion of glucose data varying in a wide range, spanning from normotolerance to overt diabetes; 3) deep shape analysis not only of glucose but also of insulin and C-peptide curves; and 4) definition of shape indices based on the whole OGTT data. Thus, our study represents a significant improvement compared with the first study on the OGTT shape (19); in that study, only 2-h, 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 focusing on OGTT shape (9), but it essentially shared the limitations of the earlier study (19).

Our results, in part, agree with what has been reported by Tschritter et al. (19): 1) 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 (19)] are associated with better glucose tolerance (i.e., lower glucose concentrations). However, probably because of the longer OGTT period, we were able to also observe a large number of glucose curves with triphasic shape, and even with 4/5-phases (three peaks), which were obviously associated with 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). These more complex shapes were observed also in the insulin and C-peptide curves, although 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, 3 h 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. The reason may be 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 toward 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 β-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 for these findings, and what regulatory mechanisms are possibly involved? Tschritter et al. (19) seems to conclude that the better metabolic condition observed in the biphasic compared with 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 β-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 a 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 β-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 Sturis et al. (18); indeed, we found higher variability in the healthier individuals. Also, the hypothesis that reduced pulsatility may be due to a β-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 β-cell function parameter of proven relevance and reliability (11). Furthermore, 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 remains unclear in our study. Regarding 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 a few main physiological defects: insulin resistance in muscle and liver and impairment in β-cell function. Recently, it has become evident that β-cell function plays a more important role than previously expected: it appears that it is the progressive impairment in β-cell function that actually determines the rate of progression toward Type 2 diabetes (5). In some recent studies, such as Hucking et al. (7), it was shown that the OGTT may not be 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 β-cell function, if appropriate methods are used (11). On the basis of the latter study (and others), we believe that use of OGTT analysis for the assessment of β-cell function is relevant and appropriate. In this study, we found that our shape index based on the C-peptide OGTT data, WHOShCP, may, in fact, have clinical relevance in the assessment of β-cell function. It can reveal differences in β-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, WHOShCP requires only C-peptide data, and it is easy to be computed (under the condition of discrete, not continuous data, derivatives reduce simply to differences). Thus, unlike 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, WHOShCP should be more reliable than other empirical β-cell function parameters from the OGTT, such as the insulinogetic index, which is hardly capable of discriminating between subtle differences in β-cell function and is known to be prone to outliers and even negative values (20), as was found also in the data set from this study (not shown). A simplified version of WHOShCP was also computed, using data only from the samples of the traditional 2-h 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 WHOShCP may be applied in the clinical contest for simple estimation of β-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 data set. In fact, the analyzed data set was very rich (3 h of 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 data set also included some women without former gestational diabetes. Furthermore, previous studies indicate that in women with former gestational diabetes (normotolerant after delivery), compared with 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 the results presented in this study to be strongly affected by the presence of former gestational diabetes. Another issue is the fact that the data set included only women, thus excluding the possibility of drawing conclusions about possible sex-related differences. However, we subsequently analyzed a small data set of 2-h OGTT data, including both females ($n = 49$) and males ($n = 23$) with glucose tolerance spanning from normoglycemia to overt diabetes (not reported in RESULTS). In this data set, females and males had, on average, similar age, BMI, and glycemic concentrations, and they showed no significant difference in any of the shape indices ($P = 0.09 – P = 0.4$), thus suggesting that sex 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 data set was small (around 10% only). However, it was found that a large majority of them was characterized by monophasic glucose shape (see Table 1). Thus, even if the number of diabetic subjects were increased, it would probably be reflected mainly in other monophasic cases, and this shape group was already well represented in our study ($n = 262$). Hence, adding 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 1) OGTT curves can be characterized by high variability, i.e., complex shape; 2) the type of shape of the glucose curve tends to be mirrored by similar shape in the insulin and C-peptide curve; 3) complex shape is associated with better glucose tolerance condition; and 4) a new shape index based on C-peptide may be viewed as a simple empirical index of β-cell function that can be easily used in clinical settings.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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