BEYOND THE MORPHOLOGY OF THE GLUCOSE CURVE FOLLOWING AN ORAL GLUCOSE TOLERANCE TEST IN OBESE YOUTH

1GIUSEPPE NOLFE, PHD
2MARIA RITA SPREGHINI PHD
2RITA WIEETRZGCWSKA SFORZA, MD
2GIUSEPPE MORINO, MD
3MELANIA MANCO, MD PHD FACN

1Institute of Cybernetics "E. Caianiello", National Research Council of Italy-CNR, Pozzuoli, 2Unit of Clinical Nutrition & 3Scientific Directorate, Bambino Gesù Hospital, IRCCS, Rome. Italy.

Running head: Shape of the OGTT curve

Key words: Children; Insulin Resistance; Insulin Secretion; Morphology; Oral Glucose Tolerance Test; Obesity; Shape.

Abbreviations: Disposition index, DI; Endogenous Glucose Production, EGP; Hepatic Insulin Resistance Index, HIRI; Homeostasis Model Assessment of Insulin Resistance, HOMA-IR; 1-Hour Plasma Glucose, 1HPG; Impaired Fasting Glucose, IFG; Impaired Glucose Tolerance, IGT; Insulino-Genic Index, ISSI-2, Insulin Secretion-Sensitivity Index 2; IGI; Insulin Sensitivity Index, ISI; Glucose Tolerance at the peak, GT_p; Muscle Insulin Sensitivity, MISI; Oral Glucose Insulin Sensitivity, OGIS; Normal Glucose Tolerance, NGT.

The authors have nothing to declare. All authors have directly participated in the execution and analysis of the study. All authors are aware of and agree to the content of the manuscript, and they have approved the final version submitted and their being listed as an author on the manuscript. The contents of this manuscript have not been copyrighted or published previously, except for the abstract which was presented at the 47th meeting of the European Association for the study of Diabetes. The contents of this manuscript are not now under consideration for publication elsewhere. The submitted manuscript nor any similar manuscript, in whole or in part, will be neither copyrighted, submitted, or published elsewhere while under consideration in the EJE.

Corresponding Author:
Melania Manco, MD PhD
Scientific Directorate
Bambino Gesù Hospital
Piazza SanOnofrio 4
I-00165, Rome.
e-mail: melania.manco@opbg.net

Copyright © 2011 European Society of Endocrinology.
ABSTRACT WORD COUNT: 250
TEXT WORD COUNT: 3,283
REFERENCES: 22
TABLES: 2
FIGURES: 3
SUPPLEMENTAL TABLES: 2
ABSTRACT

BACKGROUND To describe morphology of glucose curve during the oral glucose tolerance test (OGTT) and any association with glucose tolerance, insulin action and secretion in obese youth.

STUDY DESIGN Cross-sectional.

METHODS OGTT data of 553 patients were analysed. Subjects were divided in groups based on morphology (i.e., monophasic, biphasic, triphasic and upward monotonous) of the glucose curve. Insulin action was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR), the insulin sensitivity (ISI), the muscular insulin sensitivity (MISI) and the hepatic insulin resistance (HIRI) indexes, and the oral glucose insulin sensitivity (OGIS). Insulin secretion was estimated by the insulino-genic index (IGI). Disposition index (DI), including the Insulin-Secretion-Sensitivity Index-2 (ISSI-2), and Areas under glucose (AUC\(_G\)) and insulin (AUC\(_I\)) curves were computed.

RESULTS In patients with normal glucose tolerance (N=522), prevalent morphology of the glucose curve was monophasic (N=285, 54%). Monophasic morphology was associated with the highest concentration of 1-hour plasma glucose (1HPG, p<0.0001) and AUC\(_G\) (p<0.0001); biphasic morphology with better insulin sensitivity as estimated by OGIS (p<0.03) and lower AUC\(_I\) (p<0.0001); triphasic morphology with the highest values of HIRI (p<0.02) and IGI (p<0.007).

By combining morphologies of glucose and insulin curves or time of the glucose peak, a deeper characterization of different phenotypes of glucose metabolism emerged.

CONCLUSIONS Morphologies of the glucose curve seem reflecting different metabolic phenotypes of insulin action and secretion, particularly when combined with morphologies of insulin curve or time of glucose peak. Such findings may deserve validation in cohort study, in which glucose metabolism would be estimated by using gold-standard techniques.
**INTRODUCTION**

Altered glucose metabolism and type 2 diabetes mellitus enclose a group of metabolic diseases characterized by altered glycaemia resulting from defects in insulin secretion, action (insulin resistance increased in the skeletal muscle and/or liver) or both (1).

Surrogate measures of insulin action and secretion can be obtained from plasma glucose and insulin during the OGTT (1). Such indexes can be helpful for deeper phenotyping of individuals with altered glucose metabolism and tracking the natural history of the disease. During the OGTT, insulin action and secretion would modulate rate of plasma glucose increase and decrease, and time required for plasma glucose to peak and to return to fasting levels (2). First-phase insulin secretion and hepatic insulin resistance are important determinant of the initial rise of plasma glucose following the glucose ingestion. The rate of decline in plasma glucose concentration back toward the fasting levels seems to depend prevalently on late-phase insulin secretion and muscle insulin sensitivity (3). In that, the shape that the glucose curve assumes during the OGTT results from the net and dynamic balance between insulin action and secretion.

Few studies have attempted to answer the question of whether the shape of the glucose curve over the time following the OGTT predicts the risk of altered glucose metabolism and harbours metabolic information not captured by the level of glycaemia alone (4-7). Findings of such studies appear to be still inconsistent.

In the present study, we aim to describe the morphology of the glucose curve following the glucose load in a sample of Caucasian obese children and adolescents, together with morphologies of the insulin curve and time of glucose peaking. As researchers did in the past (4-7), we speculate that different morphologies of the glucose and insulin curves may resemble differences in insulin action and secretion.
METHODS

Data from 553 Caucasian youngsters were retrospectively analysed. Patients were consecutively referred for overweight by general paediatricians to the Unit of Clinical Nutrition at the “Bambino Gesù” Hospital from January 2008 to December 2010. Inclusion criteria were age ranging from 4 to 18 years; obesity (BMI≥95th percentile for age and sex) (8) with no previous treatment for obesity, no systemic and endocrine disease, no previous diagnosis of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diabetes (9), no use of medication, alcohol or recreational drugs. Weight and height were measured following standard procedures. The BMI z-score was calculated using US reference values (8).

The study protocol conformed to the guidelines of the European Convention of Human Rights and Biomedicine for Research in Children and to those of the Ethics Committee of the “Bambino Gesù” Hospital.

Oral glucose tolerance testing

Glucose tolerance was classified according to the criteria of the American Diabetes Association classification (9). A standard OGTT (1.75 g/kg body weight up to 75 g) was performed with flavoured glucose (Glucosio Sclavo Diagnostics, 75g/150 ml) after 8-10 hour overnight fast. After local application of an aesthetic cream (EMLA Cream), one ante-cubital i.v. catheter was inserted for blood sampling and was maintained patent by a normal saline drip during the test. Two baseline samples were obtained at -15’ and 0’ followed by sampling at 30’, 60’, 90’ and 120’ for measurements of plasma glucose and insulin.

A parameter of glucose tolerance (10) was calculated taking into account the glucose peak

\[ G_p[mg \cdot dl^{-1}] \]

irrespective of the time when the peak is reached: \( GT_p = \frac{10^6}{G_p(G_p - 70)} \). This parameter may have a more significant meaning than the glucose levels at arbitrarily chosen intervals of 60’, 90’ or 120’, as the glucose peak may occur at different times in each individual, and combines peripheral insulin activity and beta-cell secretory function (10).

The Homeostasis Model Assessments of fasting Insulin Resistance was computed according to Matthews’(11):

\[ HOMA-IR = \frac{I_0 \cdot G_0}{22.5} \]

with glucose and insulin expressed in \( mmol \cdot l^{-1} \) and \( \mu UI \cdot ml^{-1} \). The Insulin sensitivity Index (12), \( ISI = \frac{10^4}{(G_0 \cdot I_0 \cdot m_c \cdot m_i)^2} \), where \( m_c \) and \( m_i \) are the averaged glucose insulin concentration, and OGIS, the Oral Glucose Insulin Sensitivity (13), were both computed with glucose expressed in \( mg \cdot dl^{-1} \) and insulin measured in \( \mu UI \cdot ml^{-1} \). We also
estimated hepatic insulin resistance (HIRI) by \( AUC_{g0-30} \times AUC_{i0-30} \), where \( AUC \) is the area under curve of glucose and insulin, respectively, in the interval between 0’ and 30’. AUCs were estimated using the trapezoidal integration rule and with glucose, insulin and time expressed in \( mg \cdot dl^{-1} \), \( \mu UI \cdot ml^{-1} \) and minutes, respectively. The skeletal muscle insulin sensitivity (MISI) was evaluated, with the aforementioned units of measurements, by \( \frac{1}{m_i} \cdot \frac{\Delta G}{\Delta t} \), where \( \Delta G \) is the plasma glucose decay from its peak to its nadir during the OGTT (3).

The insulin secretion was estimated by means of the following parameters: the insulinogenic index (17) defined as

\[
IGI = \frac{I_{30} - I_0}{G_{30} - G_0} \left[ \frac{\mu UI \cdot ml^{-1}}{mmol \cdot l^{-1}} \right],
\]

and the ratio \( \frac{AUC_I}{AUC_G} \).

The disposition index was calculated as the product of IGI and ISI (DI\textsubscript{ISI}), OGIS (DI\textsubscript{OGIS}), or \( \frac{AUC_I}{AUC_G} \) (termed as Insulin Secretion-Sensitivity Index-2, SSI-2) (18).

**Morphologies of the curves**

Subjects were divided in groups based on morphologies of glucose and insulin curves and peak-time of glucose and insulin during the OGTT.

Briefly, the glucose or insulin concentration increases gradually and reaches its maximum after two hours in those subjects classified as having a “Monotonous” curve. Plasma glucose reaches its maximum value between 30’ and 90’ and then decreases in subjects with “monophasic” curve. The levels of glucose at 90’ must not be less than that at 120' and, in detail,

\[ G_{120} - G_{90} < +4.5mg \cdot dl^{-1}, \]

where \( 4.5mg \cdot dl^{-1} \) is the measurement error for plasma glucose.

In the biphasic curve, the peak of the curve may occur at 30’ or 60’, but between 90’ and 120’ still goes significantly up: \( G_{120} - G_{90} > +4.5mg \cdot dl^{-1} \). If the maximum is reached at 30’, we can also observe a slope between 60’-120’:

\[ G_{120} - G_{60} > +4.5mg \cdot dl^{-1}. \]

In some rare cases, a biphasic curve may present with the glucose peak at 120’.

The triphasic curve is characterized by double positive peaks at 30’ and 90’ and negative peak at 60’. For glucose, the following condition must be satisfied:

\[ G_{120} - G_{90} < -4.5mg \cdot dl^{-1}. \]

The same has been done in order to classify insulin curves. Different morphologies of the glucose or insulin curves are reported in Figure 1.

**Analytical methods**
Plasma glucose was immediately measured by the glucose oxidase technique (Cobas Integra; Roche, Basel, Switzerland). Insulin was measured by radioimmunoassay (MYRIA Technogenetics, Milan, Italy).

**Statistical analysis**

Continuous data are reported as mean and standard error of the mean (SEM), with categorical data as counts and percentages. Skewed data were logarithmically transformed before comparisons. \( \chi^2 \)-test was used for categorical variables, and ANOVA followed by the post-hoc Bonferroni test for comparison among groups with different time-peaks and morphologies. Spearman rank correlations were investigated. The level of significance was set at \( \alpha < 0.05 \). Data analysis was performed by using SPSS statistical software (SPSS V12.0, Inc., Chicago, IL) and MatLab (Mathworks, Natick, MA) for categorization of curve morphologies and peak-times.
RESULTS

Data of 553 patients (Table 1S) were included in the analysis. Of the studied subjects, 522 had normal glucose tolerance (NGT), whilst 31 (5.6%) met criteria for IGT. Statistically significant differences were observed between males and females and groups with different glucose tolerance (Table S2). NGT patients served for the analyses of morphology and peak-time.

Glucose and insulin peaks and curve morphologies during the OGTT in NGT individuals

Table 1 shows distributions of glucose and insulin peaks (Panel A) and curve morphologies (Panel B) following the oral test.

The glucose peak occurred most frequently at 30’ and was accompanied by the synchronous peak of insulin. No difference was observed among subjects with the glucose peak at different time-points of the OGTT (data not shown).

Prevalent morphologies of the glucose curve were monophasic (54.5%) or biphasic (33%) (Table 1, Panel B). Different morphologies of the glucose curve were associated with significant differences in BMI ($p<0.001$), FPG ($p<0.007$), $I_{30}$ ($p<0.02$), $I_{120}$ ($p<0.002$), HIRI ($p<0.02$), AUC$_I$ ($p<0.04$), OGIS ($p<0.01$), 1HPG, 2HPG, GT$_p$, MISI, AUC$_G$ and DI ($p<0.0001$ for all the latter comparisons) (Table 2).

Characterization of NGT subjects with monophasic and/or biphasic shapes of the glucose and insulin curves (Figure 2)

As most of the NGT subjects had monophasic or biphasic shapes of glucose and insulin curves, we divided subjects belonging these groups (N=387) in 4 subgroups: those having monophasic (MM group, N=220), biphasic (BB group, N=19) morphologies of both glucose and insulin curves, or monophasic glucose and biphasic insulin curves (MB group, N=110), or vice versa (BM group, N=38). The 4 groups differed significantly in BMI z-score ($p<0.01$); waist circumference ($p<0.01$); FPG ($p<0.04$), FPI ($p<0.001$), HIRI ($p<0.04$), HOMA-IR ($p<0.003$), ISI ($p<0.001$), OGIS ($p<0.04$), GT$_p$ ($p<0.01$), AUC$_I$ ($p<0.02$), ISSI-2 ($p=0.004$), and 1HPG; 2HPG; $I_{120}$, MISI, AUC$_G$ ($p<0.0001$ for all the latter comparisons).

Characterization of NGT subjects with monophasic and/or biphasic shapes of the glucose and glucose peaking at 30’ or 60’ (Figure 3)

We also investigated differences between individuals (N=454) with monophasic glucose curve and glucose peaks at 30’ (M$_{G30}$, N=278) or 60’ (M$_{G60}$, N=117), or biphasic peaking at 30’ (B$_{G30}$, N=38) or 60’ (B$_{G60}$, N=19). Differences were observed in 1HPG, HIRI, $I_{30}$, $I_{120}$ ($p<0.0001$ for all the
comparisons), 2HPG (p<0.05), AUC_G (p<0.003), AUC_I (p<0.009), OGIS (p<0.04), ISI (p<0.014), MISO (p<0.006), IGI (p<0.003), ISSI-2 (p=0.005) and DI_SI (p<0.002).

Correlation analyses in NGT subjects
The Spearman’s $r_o$ was used to evaluate the association between 1HPG, 2HPG, AUC_G and indexes of insulin sensitivity (ISI and OGIS) in the whole sample of NGT individuals and in subgroups identified by the different morphologies of the glucose curve.

In the whole sample of NGT subjects, 1HPG ($r_o=0.822$) correlated better than 2HPG ($r_o=0.563$) with AUC_G. In individuals with upward monotonous morphology, $r_o$ were 0.805 and 0.682; in those with monophasic shape 0.865 and 0.657; biphasic 0.722 and 0.522, and triphasic 0.719 and 0.780, respectively (p<0.0001 in all the cases).

The best correlations between ISI and OGIS (p<0.0001 in all the cases) were observed in subjects with triphasic ($r_o=0.745$) or monophasic morphologies ($r_o=0.688$). The correlation was modest in subjects with biphasic curve ($r_o=0.591$).

Distribution of peaks and morphologies in IGT patients
Morphology of the glucose curve was upward monotonous in 5 patients (16%), monophasic in 16 (52%) and biphasic in 10 (32%). The shape of the insulin curve was monotonous in 10 patients (32%), monophasic in 8 (26%), biphasic in 11 (35.5%) and triphasic in 2 (6%) patients. The glucose peak occurred more frequently at 60’ (N=14, 45%). Three patients (~10%) had the glucose peak at 30’, eight (26%) at 90’ and six of them at 120’ (19.4%).
**DISCUSSION**

Differences in the morphology of the glucose curve following the OGTT, named as monophasic, biphasic, triphasic and upward monotonous, were associated with significant variation of parameters related to insulin action, sensitivity and glucose tolerance. Prevalent morphologies of the glucose curve was monophasic in obese normo-tolerant young obese individuals, with glucose peaking more frequently at 30’ and 60’.

Monophasic morphology was associated with the highest values of 1HPG and the greatest AUC\(_G\), which would represent a risk factor for altered glucose metabolism (5, 15-17). Indeed, Kanauchi et al observed that monophasic morphology occurs more frequently in IGT than in NGT adult individuals (4). When patients with monophasic morphology of the glucose curve were deeper characterized by describing the morphology of the insulin curve (Figure 2), subjects with monophasic morphologies of both curves or biphasic insulin were those who exhibited greater excursion of 1HPG and AUC\(_G\). Subjects with monophasic glucose and biphasic insulin had also the greatest HOMA-IR and HIRI, which both suggest an increased basal and not adequately suppressed endogenous glucose production (EGP), respectively, following the glucose load. EGP, together with reduced muscle insulin sensitivity, may contribute toward altered glucose tolerance in these individuals. Indeed, they presented with greater values of 1HPG, 2HPG and AUC\(_G\) and a lower glucose disposition index as estimated by the ISSI-2. These subjects may belong to a class of increased risk for altered glucose metabolism because of both altered hepatic and muscle insulin sensitivity.

Conversely, patients presenting with biphasic morphology of the glucose curve and monophasic insulin had the lowest values of 1HPG, 2HPG and AUC\(_G\), with values of muscle insulin sensitivity and glucose disposition indexes in the upper range (Figure 2). Thus, they may exhibit a metabolic phenotype at lower risk for altered glucose metabolism. The biphasic shape of glucose tended to be associated with better insulin sensitivity, but this trend achieved the statistical significance only when insulin sensitivity was estimated according to Mari’s. Tschritter et al (6) noticed that individuals with biphasic morphology of the glucose curve had a more efficient disposal of the glucose concentrations to baseline.

Intriguing results came into view as well, when individuals with either monophasic (M) or biphasic (B) glucose shape were categorised according to the time when the glucose peaked, either 30’ (M\(_{G30}\) and B\(_{G30}\)) or 60’ (M\(_{G60}\) and B\(_{G60}\)). M\(_{G30}\) had the lowest values of AUC\(_G\) and the best disposition index as estimated by the ISSI-2, supporting the concept elsewhere demonstrated that the more quickly the plasma glucose
concentration returns to or below the fasting glucose level following glucose ingestion, the lower is the risk for future diabetes (2).

M_{G=60} individuals had lower levels of I_{30} and, consequently, of IGI and HIRI. B_{G=60} had the highest values of HOMA-IR and a value of HIRI in the upper levels, again suggesting altered gluconeogenesis associated with such phenotype. Reduced muscle insulin sensitivity is likely to cause increased 2HPG in B_{G=60} individuals (Figure 3).

With regard to the clinical meaning of glucose peaking at 1 hour, a growing body of literature supports the role of exaggerated 1HPG as risk factor for diabetes (15-17). Novel cut-off value for 1HPG (≥155 mg/dl) would identify a novel class of pre-diabetes, particularly when associated with features of the metabolic syndrome (15-16). In our series of individuals with monophasic glucose curve, 1HPG (β=0.546) and triglycerides (β=0.164) entered a regression model predicting 2HPG (R^2=0.32, p>0.0001). In the model, age, BMI z-score, HOMA-IR, total and HDL-cholesterol, waist circumference were excluded variables.

The triphasic morphology was associated with the lowest concentration of 2HPG, the highest of insulin at 30’, the greatest values of insulino-genic index and hepatic insulin resistance and the best disposition index. Indeed, calculation of IGI and HIRI requires insulin concentration at 30’. Individuals belonging to this group had a greater insulin release as estimated by the IGI, which, in turn, would protect them against development of diabetes. These individuals seem able to compensate fully for insulin resistance as evident by their disposition index. DI is the best predictor for altered glucose also in the youth (19). In the Kanauchi’ series (4), NGT individual presented more frequently biphasic or two-peak (termed triphasic in our series) morphologies of the curve.

Respect with previous studies (4-7), we tried to deeper phenotype obese patients by matching morphologies of the glucose curve with those of the insulin curve and characterizing them by the time of glucose peaking. We also investigated associations between morphologies and indexes of insulin action and secretion, commonly used in epidemiological settings. In one of such studies, an index of the morphology, associated with glucose tolerance, was developed (5). Successively, Kanauchi et al (4) investigated the association between morphology and time of glucose peaking, as we did. More recently, Abdul-Ghani et al (5) explored the relationship between morphology and rate of glucose declining toward or below preload values.

Findings from our analysis suggest that subjects with monophasic shape of the glucose curve and biphasic insulin are at increased risk for altered glucose metabolism, and, on the contrary, those presenting with biphasic glucose and monophasic insulin are at lower risk. Due to the lack of longitudinal data, our findings can provide the rationale for future confirmatory studies. Moreover, we are aware that some associations (i.e., with the AUC_G) may be expected and due to a kind of co-
linearity. Nevertheless, the suspect emerges that some formulae may adapt better to estimate insulin metabolism in individuals with certain morphology of the glucose curve and may not work efficiently in others. For instance, the OGIS model is based on the concept of a single compartment in which there is a linear association between concentration of plasma glucose and that of insulin. Moreover, it assumes that in the last hour of the OGTT, the glucose concentration exhibits a clear down slope. In some individuals (i.e., those with a biphasic or triphasic morphologies), the relationship between glucose and insulin may be perturbed by an altered milieu of gut hormones. Furthermore, in patients belonging to these categories, plasma glucose does not display a down slope as postulated in the Mari’s model.

The Matsuda’s index as well may not perform adequately in subjects with biphasic and upward monotonous glucose morphologies. The ISI-composite presupposes that the initial rise of plasma glucose concentration is mainly determined by the hepatic insulin resistance and by the suppression of EGP in response to the insulin that is secreted in response to hyperglycemia. Mean values of glucose and insulin during the OGTT would resume what happens for the subsequent 60’ to 120’, when EGP is maximally suppressed and remains constantly suppressed (20). Therefore, the rate of decline in plasma glucose concentration from its peak value to its nadir primarily reflects glucose uptake by peripheral tissues, muscle, and the insulin secretory response to hyperglycemia. Nevertheless, the intersection between insulin sensitivity and secretion may be more complex than that in some subjects in whom EGP may be not adequately suppressed. Indeed, during the OGTT the suppression of EGP is much less complete than during the euglycaemic clamp (20).

Our analysis was done with the aim at enlarging the spectrum of categories for altered glucose metabolism and providing clinicians with more simple means to estimate risk for reduced insulin action and secretion instead of mathematical formulae. Our study cannot definitively answer the question whether and to what extent different morphologies of the glucose curve reflect faithfully inter-individual differences in insulin action and secretion. To answer such question, there is need of cohort study in which both are estimated by clamp techniques and use of tracers. However, our findings support strongly that morphology, intersection of different morphologies and time of glucose peak identify different metabolic profiles of risk for altered glucose metabolism.

We are aware of several other caveats affecting our study, which enrolled young obese patients. First, HOMA-IR, ISI and IGI are the only indexes of insulin action and secretion validated so far in youth (21). No information was provided on reproducibility of the test and intra-individual variability of glucose morphology. Indeed, if patterns of glucose and insulin shapes are not reproducible, in turn, such analysis will have limited applicability.
Glucose tolerance estimated by the test may vary significantly in the same subject and its reproducibility is particularly poor among those adolescents with the greatest degree of apparent abnormality (22).
A complete analysis of the morphology would consider data on the observational interval of 180 min or longer.
We also did not provide information on several other pivotal factors, which modulate the rate of glucose increase and decrease, namely glucose absorption and gastric emptying, components of beta-cell function, release of gastro-intestinal hormones (i.e., glucagon, Glugagon-like peptide-1 and gastric inhibitory peptide) and sensitivity of their receptors for their ligands.

In conclusion, different morphologies of the glucose curve, their association with morphologies of the insulin curve and time of glucose peak may represent useful means to describe different phenotypes of glucose tolerance, insulin action and secretion in young obese individuals. An increased risk for altered glucose metabolism may be suspected in individuals with monophasic glucose but biphasic insulin or in those presenting with an exaggerated glucose peak at 1 hour. Nevertheless, cohort study estimating insulin action and secretion by using gold-standard techniques are needed to understand whether novel abnormalities identified during the OGTT, represent something valuable in the clinical practice. The OGTT is useful, in research settings, to estimate systemic insulin sensitivity and β-cell function in large population studies, but findings from our study highlight the importance of the morphology before applying common indexes of insulin action and secretion.

ACKNOWLEDGEMENTS
The authors’ responsibilities were as follows: MM & GN concept and design of the study; data analysis, drafting of the manuscript and revision for important intellectual content; MRS data collection; MRS, RWS & GM: patients’ follow-up and critical revision.

DISCLOSURE: All authors have nothing to disclose. The work was supported by a grant to MM from the Italian Ministry of Health (RF-OPG-20081142374).
REFERENCES-

LEGENDS TO FIGURES

FIGURE 1
Monophasic (right-hand) and biphasic (left-hand) morphologies of the glucose curve are depicted in the upper panels, whilst triphasic and upward monotonous are in the lower panels.

FIGURE 2
Box plots of parameters of insulin action, secretion and glucose tolerance (expressed as ln) in the group of individuals with monophasic (MM) or biphasic (BB) morphologies of both glucose and insulin curves or of monophasic glucose and biphasic insulin (MB) or vice versa (BM). ANOVA demonstrated significant differences among groups with monophasic and biphasic morphologies of the glucose and insulin curves in HOMA-IR ($p<0.001$), HIRI ($p<0.04$), 1HPG ($p<0.001$); 2HPG ($p<0.0001$); AUC$_G$ ($p<0000.1$), ISI ($p<0.001$), I$_{120}$ ($p<0.0001$), AUC$_I$ ($p<0.02$), MISI ($p<0.0001$), and ISSI-2 ($p=0.004$). Statistical significance at the post hoc test was as it follows:

HOMA-IR was the greatest in the MB group ($p$ ranging from 0.05 to 0.001). MM and MB individuals had greater 1HPG ($p<0.0001$ in all the comparisons of monophasic glucose versus biphasic); 2HPG was lower in MM individuals respect with BB patients ($p<0.0001$). AUC$_G$ was lower in BM and BB group ($p$ ranging from 0.005 to 0.001). Individuals with biphasic glucose had greater ISI than MB individuals ($p=0.001$ and 0.01, respectively). MM subjects were more sensitive to insulin than MB subjects ($p<0.007$). The glucose disposition index calculated as ISSI-2 was the lowest in MB individuals ($p=0.002$ vs. both MM and BM).

FIGURE 3
Box plots of parameters of insulin action, secretion and glucose tolerance (expressed as ln) in the group of individuals with monophasic (M) or biphasic (B) morphology of the glucose curve and plasma glucose peaking at 30’ (M$_{G30}$ or B$_{G30}$) or 60’ (M$_{G60}$ or B$_{G60}$). Differences at the ANOVA were observed in 1HPG, HIRI, I$_{30}$, I$_{120}$ ($p<0.0001$ for all the comparisons), 2HPG ($p<0.05$), AUC$_G$ ($p<0.003$), AUC$_I$ ($p<0.009$), OGIS ($p<0.04$), ISI ($p<0.014$), MISI ($p<0.006$), IGI ($p<0.003$), ISSI-2 ($p=0.005$). B$_{G60}$ had the highest value of HOMA-IR as compared with M$_{G30}$ and M$_{G60}$ ($p<0.03$ for both). M$_{G30}$ had the lowest values of AUC$_G$ as compared with M$_{G60}$ ($p<0.02$) and B$_{G30}$ ($p<0.04$). I$_{30}$ was the lowest in MG60 ($p<0.0001$ vs. M$_{G30}$ and B$_{G30}$; B$_{G30}$ vs. B$_{G60}$, $p<0.04$). HIRI was the lowest in M$_{G60}$ vs. M$_{G30}$ and B$_{G30}$ ($p<0.0001$). M$_{G30}$ and M$_{G60}$ differed significantly in IGI ($p<0.01$).

The best disposition index as calculated by ISSI-2 was observed in M$_{G30}$ individuals ($p=0.03$ vs. B$_{G30}$ and $p=0.004$ vs. B$_{G60}$ )
Table 1 Distribution of the glucose and insulin peaks (Panel A) and morphologies (Panel B) following the glucose load in NGT obese patients.

<table>
<thead>
<tr>
<th>Panel A</th>
<th>Time of the glucose peak</th>
<th>Minutes</th>
<th>30’</th>
<th>60’</th>
<th>90’</th>
<th>120’</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of the insulin peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30’</td>
<td></td>
<td>238</td>
<td>19</td>
<td>13</td>
<td>7</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>(45.6%)</td>
<td></td>
<td>(3.6%)</td>
<td>(2.5%)</td>
<td>(1.3%)</td>
<td>(53%)</td>
<td></td>
</tr>
<tr>
<td>60’</td>
<td>48</td>
<td></td>
<td>78</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>(9.2%)</td>
<td></td>
<td>(14.9%)</td>
<td>(0.8%)</td>
<td>(1.1%)</td>
<td>(26%)</td>
<td></td>
</tr>
<tr>
<td>90’</td>
<td>17</td>
<td></td>
<td>27</td>
<td>13</td>
<td>3</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3.2%)</td>
<td></td>
<td>(5.2%)</td>
<td>(2.5%)</td>
<td>(0.6%)</td>
<td>(11.5%)</td>
<td></td>
</tr>
<tr>
<td>120’</td>
<td>14</td>
<td></td>
<td>13</td>
<td>7</td>
<td>15</td>
<td>49 (9.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.9%)</td>
<td></td>
<td>(2.5%)</td>
<td>(1.3%)</td>
<td>(2.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>317</td>
<td></td>
<td>137</td>
<td>37</td>
<td>31</td>
<td>522</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(60.5%)</td>
<td></td>
<td>(26.2%)</td>
<td>(7%)</td>
<td>(5.9%)</td>
<td>(100%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Panel B</th>
<th>Morphology of the Glucose Curve</th>
<th>Monotonous</th>
<th>Monophasic</th>
<th>Biphasic</th>
<th>Triphasic</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphology of the Insulin Curve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monotonous</td>
<td>9</td>
<td>11</td>
<td>1</td>
<td>-</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(1.72%)</td>
<td>(2.1%)</td>
<td>(0.2%)</td>
<td>(0.2%)</td>
<td>(4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monophasic</td>
<td>7</td>
<td>192</td>
<td>32</td>
<td>7</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>(1.3%)</td>
<td>(36.8%)</td>
<td>(6.1%)</td>
<td>(1.3%)</td>
<td>(45.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biphasic</td>
<td>5</td>
<td>58</td>
<td>133</td>
<td>8</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>(0.95%)</td>
<td>(11.1%)</td>
<td>(25.4%)</td>
<td>(1.5%)</td>
<td>(39%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triphasic</td>
<td>-</td>
<td>24</td>
<td>6</td>
<td>29</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4.6%)</td>
<td>(1.1%)</td>
<td>(5.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>21</td>
<td>285</td>
<td>172</td>
<td>44</td>
<td>522</td>
</tr>
<tr>
<td></td>
<td>(4%)</td>
<td>(54.5%)</td>
<td>(32.9%)</td>
<td>(8.4%)</td>
<td>(100%)</td>
<td></td>
</tr>
<tr>
<td>Morphology of the Glucose Curve</td>
<td>Monotonous</td>
<td>Monophasic</td>
<td>Biphasic</td>
<td>Triphasic</td>
<td>p&lt;</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>285</td>
<td>172</td>
<td>44</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/11</td>
<td>146/139</td>
<td>98/74</td>
<td>26/18</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.5±0.47</td>
<td>11.2±0.16</td>
<td>10.7±0.21</td>
<td>10.4±0.41</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.9±1.24</td>
<td>28.9±0.3</td>
<td>27.3±0.31</td>
<td>28.12±0.7</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>BMI z-score (SDS)</td>
<td>2.24±0.08</td>
<td>2.22±0.02</td>
<td>2.12±0.04</td>
<td>2.27±0.06</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>87±2</td>
<td>84.3±0.82</td>
<td>82±0.85</td>
<td>82.6±2.26</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.51±0.36</td>
<td>5.42±0.08</td>
<td>5.15±0.09</td>
<td>5.51±0.32</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>153.3±5.2</td>
<td>154±1.81</td>
<td>154.4±3</td>
<td>160.2±4.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>48.9±2.3</td>
<td>53.3±0.86</td>
<td>53.2±1.19</td>
<td>51±1.36</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>86.8±4.9</td>
<td>85.2±1.55</td>
<td>85.6±1.95</td>
<td>92.4±4.25</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>87.9±7.6</td>
<td>82.8±2.1</td>
<td>78.8±2.65</td>
<td>86.490</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>ALT (UI/ml)</td>
<td>23.3±1.82</td>
<td>24.5±0.79</td>
<td>23.5±0.53</td>
<td>24.6±1.17</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>AST (UI/ml)</td>
<td>26±3.41</td>
<td>26.1±1.64</td>
<td>24.1±1.07</td>
<td>23.9±1.81</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>69.9±1.52</td>
<td>73.5±0.43</td>
<td>75.3±0.58</td>
<td>74.3±1.31</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>1HPG (mg/dl)</td>
<td>104.1±1.81</td>
<td>129±1.3</td>
<td>112.1±1.50</td>
<td>97.0±2.48</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>2HPG (mg/dl)</td>
<td>116.1±2.19</td>
<td>105.3±0.91</td>
<td>112.9±0.89</td>
<td>100.8±2.37</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>I₀ (µUI/ml)</td>
<td>18.7±3.5</td>
<td>14.6±0.72</td>
<td>13.4±0.85</td>
<td>13.1±0.87</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>I₃₀ (µUI/ml)</td>
<td>89.5±22.6</td>
<td>114.3±4.58</td>
<td>120.3±5.86</td>
<td>147.7±13.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>I₁₂₀ (µUI/ml)</td>
<td>126.5±19.6</td>
<td>17.6±3.3</td>
<td>77.3±3.4</td>
<td>64.02±7.01</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.28±0.65</td>
<td>2.67±0.12</td>
<td>2.52±0.16</td>
<td>2.44±0.17</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>OGIS ‡</td>
<td>469±21.37</td>
<td>479.2±4.8</td>
<td>499.8±5.5</td>
<td>475.4±13.35</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>ISI</td>
<td>4.74±0.66</td>
<td>4.66±0.25</td>
<td>5.14±0.33</td>
<td>4.38±0.30</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>HIRI</td>
<td>4.11±0.94</td>
<td>6.19±0.26</td>
<td>6.43±0.32</td>
<td>7.61±0.70</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>MISI ‡‡‡§</td>
<td>0.004±0.002</td>
<td>0.54±0.02</td>
<td>0.38±0.02</td>
<td>0.49±0.05</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>GTp</td>
<td>1.75±0.14</td>
<td>0.99±0.05</td>
<td>1.37±0.36</td>
<td>1.15±0.11</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>IGI</td>
<td>15.89±19.41</td>
<td>31.83±1.46</td>
<td>32.3±2.81</td>
<td>46.18±4.72</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>AUC₉G ‡‡‡‡</td>
<td>101.15±1.52</td>
<td>116.7±0.86</td>
<td>109.9±0.91</td>
<td>108.6±1.87</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>AUC₁ ‡</td>
<td>93.66±18.5</td>
<td>91.74±3.05</td>
<td>77.52±3.12</td>
<td>88±6.90</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>AUC₀/AUC₉G</td>
<td>1.68±0.22</td>
<td>1.67±0.06</td>
<td>1.85±0.10</td>
<td>1.59±0.13</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>DI_{ISI}</td>
<td>121.8±31.8</td>
<td>117.3±8.07</td>
<td>117.6±25.3</td>
<td>177±19.7</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>ISSI-2</td>
<td>2.96±0.31</td>
<td>2.87±0.13</td>
<td>2.88±0.11</td>
<td>2.94±0.12</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM. Statistical significance in the column refers to the ANOVA test followed by the Bonferroni post-hoc-comparison (on logarithmically transformed variables before analysis) among patients presenting with different morphologies of the glucose curves as follows:

* Monotonous vs. Monophasic (BMI, p<0.03; 1HPG, p<0.0001; 2HPG, p<0.004; MISI, p<0.0001; AUC_{G}, p<0.0001);
† Monotonous vs. Biphasic (BMI, p<0.0001; FPG, p<0.01; MISI, p<0.0001; AUC_{G}, p<0.027);
‡ Monotonous vs. Triphasic (BMI, p<0.016; 2HPG, p<0.0001; I_{30}, p<0.03; MISI, p<0.0001; IGI, p<0.005; HIRI, p<0.016);
§ Monophasic vs. Biphasic (BMI, p<0.004; 1HPG, p<0.0001; MISI, p<0.0001; 2HPG, p<0.0001; AUC_{G}, p<0.0001, AUC_{I}, p<0.02; OGIS, p<0.05);
¶ Monophasic vs. Triphasic (1HPG, p<0.0001; IGI, p<0.06; AUC_{G}, p<0.001);
³ Biphasic vs. Triphasic (1HPG, p<0.0001).
Figure 1.
Monophasic (right-hand) and biphasic (left-hand) morphologies of the glucose curve are depicted in the upper panels, whilst triphasic and upward monotonous are in the lower panels.

197x148mm (72 x 72 DPI)
Figure 2.
Box plots of parameters of insulin action, secretion and glucose tolerance (expressed as ln) in the group of individuals with monophasic (MM) or biphasic (BB) morphologies of both glucose and insulin curves or of monophasic glucose and biphasic insulin (MB) or vice versa (BM).
ANOVA demonstrated significant differences among groups with monophasic and biphasic morphologies of the glucose and insulin curves in HOMA-IR (p<0.001), HIRI (p<0.04), 1HPG (p<0.0001); 2HPG (p<0.0001); AUCG (p<0.0000.1), ISI (p<0.0011), I120 (p<0.00001), AUCI (p<0.02), MISI (p<0.00001), and ISSI-2 (p=0.004). Statistical significance at the post hoc test was as it follows:
HOMA-IR was the greatest in the MB group (p ranging from 0.05 to 0.001). MM and MB individuals had greater 1HPG (p<0.0001 in all the comparisons of monophasic glucose versus biphasic); 2HPG was lower in MM individuals respect with BB patients (p<0.0001). AUCG was lower in BM and BB group (p ranging from 0.005 to 0.001). Individuals with biphasic glucose had greater ISI than MB individuals (p= 0.001 and 0.01, respectively). MM subjects were more sensitive to insulin than MB subjects (p<0.007). The glucose disposition index calculated as ISSI-2 was the lowest in MB individuals (p=0.002 vs. both MM and BM).
Figure 3.
Box plots of parameters of insulin action, secretion and glucose tolerance (expressed as ln) in the group of individuals with monophasic (M) or biphasic (B) morphology of the glucose curve and plasma glucose peaking at 30' (MG30 or BG30) or 60' (MG60 or BG60). Differences at the ANOVA were observed in 1HPG, HIRI, I30, I120 (p<0.0001 for all the comparisons), 2HPG (p<0.05), AUCG (p<0.003), AUCI (p<0.009), OGIS (p<0.04), ISI (p<0.014), MISI (p<0.006), IGI (p<0.003), ISSI-2 (p=0.005). BG60 had the highest value of HOMA-IR as compared with MG30 and MG60 (p<0.03 for both). MG30 had the lowest values of AUCG as compared with MG60 (p<0.02) and BG30 (p<0.04).
I30 was the lowest in MG60 (p<0.0001 vs. MG30 and BG30; BG30 vs. BG60, p<0.04). HIRI was the lowest in MG60 vs. MG30 and BG30 (p<0.0001). MG30 and MG60 differed significantly in IGI (p<0.01). The best disposition index as calculated by ISSI-2 was observed in MG30 individuals (p=0.03 vs. BG30 and p=0.004 vs. BG60).