CLINICAL STUDY

Beyond the morphology of the glucose curve following an oral glucose tolerance test in obese youth

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Abstract

Background: To describe the 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 the morphology (i.e. monophasic, biphasic, triphasic and upward monotonous) of glucose curve. Insulin action was estimated by the homeostasis model assessment of insulin resistance, the insulin sensitivity, the muscle insulin sensitivity and the hepatic insulin resistance indexes (HIRI), and the oral glucose insulin sensitivity (OGIS). Insulin secretion was estimated by the insulinogenic index (IGI). Disposition index, including the insulin secretion–sensitivity index-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 h plasma glucose ($P < 0.0001$) 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 characterisation 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.

European Journal of Endocrinology 166 107–114

Introduction

Altered glucose metabolism and type 2 diabetes mellitus enclose a group of metabolic diseases characterised 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 oral glucose tolerance test (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 the rate of increase and decrease in plasma glucose, and time required for plasma glucose to peak and to return to the fasting levels (2). First-phase insulin secretion and hepatic insulin resistance index (HIRI) are important determinants of the initial rise of plasma glucose following glucose ingestion. The rate of decline

in plasma glucose concentration back towards 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 this 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.

Materials and 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 (body mass index (BMI) ≥ 95 th percentile for age and sex) (8) with no previous treatment for obesity, no systemic and endocrine disease, no previous diagnosis of impaired fasting glucose, impaired glucose tolerance (IGT) or diabetes (9), and 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, Sovicille, SI, Italy 75 g/150 ml) after 8–10 h 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/dl) irrespective of the time when the peak is reached:

$$GT_p = \frac{10^6}{G_p^2 (G_p - 70)}.$$

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 β -cell secretory function (10).

The homeostasis model assessments of fasting insulin resistance (HOMA-IR) was computed according to Matthews *et al.* (11): $HOMA - IR = (I_0 - G_0)/22.5$, with glucose and insulin expressed as mmol/l and μ UI/ml respectively. The insulin sensitivity index (ISI) (12),

$$ISI = \frac{10^4}{(G_0 \times I_0 \times m_G \times m_I)^{1/2}}$$

where m_G and m_I are the averaged glucose insulin concentration, and the oral glucose insulin sensitivity (OGIS) (13), were both computed with glucose expressed as mg/dl and insulin measured in μ UI/ml respectively. We also estimated the HIRI by $AUC_{G0-30} \times AUC_{I0-30}$, where AUC is the area under the 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 as mg/dl, μ UI/ml and minutes respectively. The skeletal muscle insulin sensitivity (MISI) was evaluated with the aforementioned units of measurements using the formula

$$\frac{1}{m_I} \times \frac{\Delta G}{\Delta t}$$

where ΔG 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 (IGI) (14) defined as

$$IGI = \frac{I_{30} - I_0}{G_{30} - G_0} \left[\frac{\mu\text{UI/ml}}{\text{mmol/l}} \right],$$

$$\text{and the ratio } \frac{AUC_I}{AUC_G}.$$

The disposition index (DI) was calculated as the product of IGI and ISI (DI_{ISI}), OGIS (DI_{OGIS}) or AUC_I/AUC_G (termed as insulin secretion-sensitivity index-2 (ISSI-2)) (15).

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 2 h 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, $G_{120} - G_{90} < +4.5$ mg/dl, where 4.5 mg/dl 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 $120'$, it still goes significantly up: $G_{120} - G_{90} > +4.5$ mg/dl. If the maximum is reached at $30'$, we can also observe a slope between $60'$ and $120'$: $G_{120} - G_{60} > +4.5$ mg/dl in some rare cases, a biphasic curve may present with the glucose peak at $120'$.

The triphasic curve is characterised by double positive peaks at $30'$ and $90'$ and negative peak at $60'$. For glucose, the following condition must be satisfied: $G_{120} - G_{90} < -4.5$ mg/dl.

The same has been done in order to classify insulin curves.

Different morphologies of the glucose or insulin curves are reported in Fig. 1.

Analytical methods

Plasma glucose was immediately measured by the glucose oxidase technique (Cobas Integra; Roche). Insulin was measured by RIA (MYRIA Technogenetics, Milan, Italy).

Statistical analysis

Continuous data are reported as mean and S.E.M., with categorical data as counts and percentages. Skewed data were logarithmically transformed before comparisons.

χ^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. The 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, USA) and MatLab (Mathworks, Natick, MA, USA) for categorisation of curve morphologies and peak times.

Results

Data of 553 patients (Supplementary Table 1, see section on supplementary data given at the end of this article) 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

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.

and females and groups with different glucose tolerance (Supplementary Table 2, see section on supplementary data given at the end of this article). NGT patients served for the analyses of morphology and peak time.

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

Tables 1 and 2 shows distributions of glucose and insulin peaks and curve morphologies 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 2). 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} ($P < 0.002$), IGI ($P < 0.007$), HIRI ($P < 0.02$), AUC_I ($P < 0.04$), OGIS ($P < 0.01$), 1 h plasma glucose (1HPG), 2HPG, glucose tolerance at the peak (GT_p), MISI, AUC_G and DI ($P < 0.0001$ for all the latter comparisons; Table 3).

Characterisation of NGT subjects with monophasic and/or biphasic shapes of the glucose and insulin curves

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 four subgroups: those having monophasic (MM group, $n = 220$), biphasic (BB group, $n = 19$) morphologies of both glucose and insulin curves, monophasic glucose and biphasic insulin curves (MB group, $n = 110$) or *vice versa* (BM group, $n = 38$; Fig. 2). The four 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; and I_{120} , MISI and AUC_G ($P < 0.0001$ for all the latter comparisons).

Characterisation of NGT subjects with monophasic and/or biphasic shapes of the glucose and glucose peaking at 30' or 60'

We also investigated the 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} , $n = 19$; Fig. 3). Differences were observed in 1HPG, HIRI, I_{30} , I_{120} ($P < 0.0001$ for all the comparisons),

Table 1 Distribution of the glucose and insulin peaks following the glucose load in normal glucose tolerance obese patients.

	Time of the glucose peak (minutes)				n
	30	60	90	120	
Time of insulin peak (minutes)					
30	238 (45.6%)	19 (3.6%)	13 (2.5%)	7 (1.3%)	277 (53%)
60	48 (9.2%)	78 (14.9%)	4 (0.8%)	6 (1.1%)	136 (26%)
90	17 (3.2%)	27 (5.2%)	13 (2.5%)	3 (0.6%)	60 (11.5%)
120	14 (2.9%)	13 (2.5%)	7 (1.3%)	15 (2.9%)	49 (9.2%)
n	317 (60.5%)	137 (26.2%)	37 (7%)	31 (5.9%)	522 (100%)

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 ($P = 0.005$) and DI_{ISI} ($P < 0.002$).

patients ($\sim 10\%$) had the glucose peak at 30', eight (26%) at 90' and six of them at 120' (19.4%).

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 five patients (16%), monophasic in 16 (52%) and biphasic in ten (32%). The shape of the insulin curve was monotonous in ten patients (32%), monophasic in eight (26%), biphasic in 11 (35.5%) and triphasic in two (6%) patients. The glucose peak occurred more frequently at 60' ($n = 14$, 45%). Three

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 were 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, 16, 17, 18). Indeed, Kanauchi *et al.* (4) observed that monophasic morphology occurs more frequently in IGT than in NGT adult individuals. When patients with monophasic morphology of the glucose curve were deeper characterised by describing the morphology of the insulin curve (Fig. 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

Table 2 Morphology of the glucose and insulin curves following glucose load in normal glucose tolerance obese patients.

	Morphology of the glucose curve				n
	Monotonous	Monophasic	Biphasic	Triphasic	
Morphology of the insulin curve					
Monotonous	9 (1.72%)	11 (2.1%)	1 (0.2%)	—	21 (4%)
Monophasic	7 (1.3%)	192 (36.8%)	32 (6.1%)	7 (1.3%)	238 (45.6%)
Biphasic	5 (0.95%)	58 (11.1%)	133 (25.4%)	8 (1.5%)	204 (39%)
Triphasic	—	24 (4.6%)	6 (1.1%)	29 (5.5%)	59 (11%)
n	21 (4%)	285 (54.5%)	172 (32.9%)	44 (8.4%)	522 (100%)

Table 3 Inter-group comparison according to the morphology of the glucose curve in normal glucose tolerance individuals. Data are shown as mean $\pm$ S.E.M.

	Morphology of the glucose curve				P<
	Monotonous	Monophasic	Biphasic	Triphasic	
n	21	285	172	44	0.5
Sex (M/F)	10/11	146/139	98/74	26/18	0.54
Age (years)	12.5 $\pm$ 0.47	11.2 $\pm$ 0.16	10.7 $\pm$ 0.21	10.4 $\pm$ 0.41	0.01
BMI (kg/m ²)*,†,‡,§	31.9 $\pm$ 1.24	28.9 $\pm$ 0.3	27.3 $\pm$ 0.31	28.12 $\pm$ 0.7	0.0001
BMI z-score (SDS)	2.24 $\pm$ 0.08	2.22 $\pm$ 0.02	2.12 $\pm$ 0.04	2.27 $\pm$ 0.06	0.048
Waist circumference (cm)	87 $\pm$ 2	84.3 $\pm$ 0.82	82 $\pm$ 0.85	82.6 $\pm$ 2.26	0.2
Uric acid (mg/dl)	5.51 $\pm$ 0.36	5.42 $\pm$ 0.08	5.15 $\pm$ 0.09	5.51 $\pm$ 0.32	0.1
Total cholesterol (mg/dl)	153.3 $\pm$ 5.2	154 $\pm$ 1.81	154.4 $\pm$ 3	160.2 $\pm$ 4.4	0.7
HDL-cholesterol (mg/dl)	48.9 $\pm$ 2.3	53.3 $\pm$ 0.86	53.2 $\pm$ 1.19	51 $\pm$ 1.36	0.4
LDL-cholesterol (mg/dl)	86.8 $\pm$ 4.9	85.2 $\pm$ 1.55	85.6 $\pm$ 1.95	92.4 $\pm$ 4.25	0.4
Triglycerides (mg/dl)	87.9 $\pm$ 7.6	82.8 $\pm$ 2.1	78.8 $\pm$ 2.65	86 $\pm$ 4.90	0.4
ALT (U/ml)	23.3 $\pm$ 1.82	24.5 $\pm$ 0.79	23.5 $\pm$ 0.53	24.6 $\pm$ 1.17	0.8
AST (U/ml)	26 $\pm$ 3.41	26.1 $\pm$ 1.64	24.1 $\pm$ 1.07	23.9 $\pm$ 1.81	0.8
FPG (mg/dl) [†]	69.9 $\pm$ 1.52	73.5 $\pm$ 0.43	75.3 $\pm$ 0.58	74.3 $\pm$ 1.31	0.006
1HPG (mg/dl)*,†,‡,§	104.1 $\pm$ 1.81	129 $\pm$ 1.3	112.1 $\pm$ 1.50	97.0 $\pm$ 2.48	0.0001
2HPG (mg/dl)*,‡	116.1 $\pm$ 2.19	105.3 $\pm$ 0.91	112.9 $\pm$ 0.89	100.8 $\pm$ 2.37	0.0001
I ₀ (μ U/ml)	18.7 $\pm$ 3.5	14.6 $\pm$ 0.72	13.4 $\pm$ 0.85	13.1 $\pm$ 0.87	0.4
I ₃₀ (μ U/ml)	89.5 $\pm$ 22.6	114.3 $\pm$ 4.58	120.3 $\pm$ 5.86	147.7 $\pm$ 13.02	0.02
I ₁₂₀ (μ U/ml)	126.5 $\pm$ 19.6	17.6 $\pm$ 3.3	77.3 $\pm$ 3.4	64.02 $\pm$ 7.01	0.002
HOMA-IR	3.28 $\pm$ 0.65	2.67 $\pm$ 0.12	2.52 $\pm$ 0.16	2.44 $\pm$ 0.17	0.4
OGIS [‡]	469 $\pm$ 21.37	479.2 $\pm$ 4.8	499.8 $\pm$ 5.5	475.4 $\pm$ 13.35	0.03
ISI	4.74 $\pm$ 0.66	4.66 $\pm$ 0.25	5.14 $\pm$ 0.33	4.38 $\pm$ 0.30	0.6
HIRI	4.11 $\pm$ 0.94	6.19 $\pm$ 0.26	6.43 $\pm$ 0.32	7.61 $\pm$ 0.70	0.02
MISI*,†,‡,§	0.004 $\pm$ 0.002	0.54 $\pm$ 0.02	0.38 $\pm$ 0.02	0.49 $\pm$ 0.05	0.0001
GT _p	1.75 $\pm$ 0.14	0.99 $\pm$ 0.05	1.37 $\pm$ 0.36	1.15 $\pm$ 0.11	0.4
IGI	15.89 $\pm$ 19.41	31.83 $\pm$ 1.46	32.3 $\pm$ 2.81	46.18 $\pm$ 4.72	0.007
AUC _G *,†,‡,§	101.15 $\pm$ 1.52	116.7 $\pm$ 0.86	109.9 $\pm$ 0.91	108.6 $\pm$ 1.87	0.0001
AUC [‡]	93.66 $\pm$ 18.5	91.74 $\pm$ 3.05	77.52 $\pm$ 3.12	88 $\pm$ 6.90	0.03
AUC _i /AUC _G	1.68 $\pm$ 0.22	1.67 $\pm$ 0.06	1.85 $\pm$ 0.10	1.59 $\pm$ 0.13	0.3
DI _{ISI}	121.8 $\pm$ 31.8	117.3 $\pm$ 8.07	117.6 $\pm$ 25.3	177 $\pm$ 19.7	0.0001
ISSI-2	2.96 $\pm$ 0.31	2.87 $\pm$ 0.13	2.88 $\pm$ 0.11	2.94 $\pm$ 0.12	0.6

FPI, fasting plasma insulin; FPG, fasting plasma glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase

Statistical significance 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, $P < 0.0001$; I₃₀, $P < 0.03$; 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, $P < 0.001$); *Biphasic vs triphasic (1HPG, $P < 0.0001$).

individuals. Indeed, they presented with greater values of 1HPG, 2HPG and AUC_G and a lower glucose DI 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 DIs in the upper range (Fig. 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 model. Tschrutter *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 at 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 DI as estimated by the ISSI-2, supporting the concept demonstrated elsewhere 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_{G60} individuals had lower levels of I₃₀ and, consequently, of IGI and HIRI. B_{G60} 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_{G60} individuals (Fig. 3).

With regard to the clinical meaning of glucose peaking at 1 h, a growing body of literature supports

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 ($P < 0.0001$), AUC_G ($P < 0.0001$), 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 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 vs biphasic); 2HPG was lower in MM individuals compared with BB patients ($P < 0.0001$). AUC_G was lower in BM and BB groups (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).

the role of exaggerated 1HPG as risk factor for diabetes (16, 17, 18). 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 (17, 18). In our group of individuals with monophasic glucose curve, 1HPG ($\beta = 0.546$) and triglycerides ($\beta = 0.164$) 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 and 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 IGI and HIRI and the best DI. 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 to be able to compensate fully for insulin resistance as evident by their DI. DI is the best predictor for altered glucose also in the youth (19). In the study by Kanauchi *et al.* (4), NGT individual presented more frequently

biphasic or two-peak (termed triphasic in our study) morphologies of the curve.

With respect to 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 characterising 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 such study, 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. Owing 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 could be due to co-linearity. Nevertheless, the suspect emerges that some formulae may adapt better to estimate insulin metabolism in individuals with certain

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, 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 ($P < 0.003$) and ISSI-2 ($P = 0.005$). BG₆₀ had the highest value of HOMA-IR compared with MG₃₀ and MG₆₀ ($P < 0.03$ for both). MG₃₀ had the lowest values of AUC_G compared with MG₆₀ ($P < 0.02$) and BG₃₀ ($P < 0.04$). I_{30} 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).

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 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 Mari's model.

Matsuda's index may also 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 HIRI and by the suppression of EGP in response to the insulin that is secreted in response to hyperglycaemia. Mean values of glucose and insulin during the OGTT would resume what happens for the subsequent 60'–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 hyperglycaemia. Nevertheless, the intersection between insulin sensitivity and secretion may be more complex than that in some subjects in whom EGP may not be 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 of 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 a question, there is need for a cohort study in which both are estimated by clamp techniques and use of tracers. However, our findings strongly support 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 that was performed on 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 β -cell function, release of gastro-intestinal hormones (i.e. glucagon, glucagon-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 h.

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.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-11-0827>.

Declaration of interest

The authors have nothing to declare. The contents of this manuscript have not been copyrighted or published previously, except as the abstract, which was presented at the 47th meeting of the European Association for the Study of Diabetes.

Funding

The work was supported by a grant to M Manco from the Italian Ministry of Health (RF-OPG-20081142374).

Author contribution statement

All authors have directly participated in the execution and analysis of the study. The authors' responsibilities are as follows: M Manco and G Nolle: concept and design of the study; data analysis, drafting of the manuscript and revision for important intellectual content; M R Spreghini: data collection; M R Spreghini, R Wietrzycowska Sforza and G Morino: patients' follow-up and critical revision.

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Received 3 August 2011

Accepted 18 October 2011