Assessing the Shape of the Glucose Curve During an Oral Glucose Tolerance Test

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OBJECTIVE — The oral glucose tolerance test (OGTT) is used to define the status of glucose tolerance based on the plasma glucose level at 120 min. The purpose of the present study was to identify parameters that determine the shape of the plasma glucose course measured at 0, 30, 60, 90, and 120 min during an OGTT.

RESEARCH DESIGN AND METHODS — OGTT data from 551 subjects (485 with normal glucose tolerance [NGT] and 66 with impaired glucose tolerance [IGT]) were analyzed. We distinguished between "monophasic," "biphasic," and unclassified glucose shapes. A "shape" index based on the extent and the direction of the plasma glucose change in the second hour allowed us to treat shape as a continuous variable.

RESULTS — In the biphasic group, the NGT-to-IGT ratio was slightly higher (173/20 vs. 209/40, $P = 0.08$) and the male-to-female ratio was lower (60/133 vs. 120/129, $P = 0.0003$). Subjects with a biphasic shape had significantly lower age, BMI, waist-to-hip ratio (WHR), HbA_{1c}, plasma glucose, and area under the insulin curve ($insulin_{AUC}$) and a better estimated insulin sensitivity and secretion (using validated indexes) than monophasic subjects (all $P < 0.05$). By adjusting this shape index for $glucose_{AUC}$ (as continuous measure of glucose tolerance), correlations with age, BMI, WHR, HbA_{1c}, and $insulin_{AUC}$ were completely abolished. The adjusted shape index was still higher in female than in male subjects but lower in IGT than in NGT subjects (both $P = 0.0003$). Finally, we tested common polymorphisms in insulin receptor substrate (IRS)-1, IRS-2, calpain-10, hepatic lipase, and peroxisome proliferator–activated receptor-γ for association with the shape index.

CONCLUSIONS — We conclude that the plasma glucose shape during an OGTT depends on glucose tolerance and sex. In addition, genetic factors seem to play a role. The shape index may be a useful metabolic screening parameter in epidemiological and genetic association studies.

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The oral glucose tolerance test (OGTT) has traditionally been used to classify the status of glucose tolerance for diagnostic purposes: normal glucose tolerance (NGT) versus impaired glucose tolerance (IGT) versus diabetes (1). More recently, however, some authors have attempted to exploit the information contained in a 2-h OGTT to estimate insulin sensitivity (2–4) and β-cell function (5). While the derived indexes are less accurate than the respective gold-standard methods, they can be obtained more easily and used in large epidemiological or genetic association studies.

These indexes take advantage of glucose and insulin concentrations at specific time points during the OGTT. To the best of our knowledge, with one exception, nobody has tried to answer the question of whether the shape of the glucose curve over time during the OGTT has any relevance. The one study addressing this issue that we found in the literature is a paper written in Japanese (with an abstract in English), where the authors classified the glucose curve during the OGTT as "biphasic," "domed," and "upward" (6). The main finding was that in patients with type 2 diabetes, the prevalence of biphasic was lower and the prevalence of upward was higher than in any other group. This appears somewhat trivial, because the category upward naturally favors enrichment with diabetic subjects who, by definition, have the highest glucose concentrations at the end of the OGTT. Nevertheless, it is interesting to note that the biphasic shape was most strongly associated with NGT in that study. This suggests that the shape harbors metabolic information not captured by the level of glycemia alone.

In the present study, we followed-up on this topic, especially on the significance of biphasic versus monophasic for the same status of glucose tolerance. For this purpose, we developed a simple index to classify the glucose curves essentially into the monophasic and the biphasic shape. In addition, this index could be treated as a continuous variable accounting for the fact that the monophasic or biphasic shape could be more or less pronounced. To detach the information contained in the shape of the glucose curve from the absolute level of glycemia (area under the glucose curve [$glucose_{AUC}$]) attained during the OGTT, we adjusted this index mathematically for the $glucose_{AUC}$. Finally, to eliminate confounding effects of metabolic extremes, such as type 2 diabetes, we restricted our analysis to nondiabetic subjects.

RESEARCH DESIGN AND METHODS

Subjects

We analyzed OGTT data of 551 Caucasian volunteers who participated in the

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Abbreviations: AUC, area under the curve; $glucose_{120}$, glucose at 120 min; $glucose_{AUC}$, area under the glucose curve; IGT, impaired glucose tolerance; IRS, insulin receptor substrate; $insulin_{AUC}$, area under the insulin curve; ISI, insulin sensitivity index; LIPC, hepatic lipase; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PPAR-γ, peroxisome proliferator–activated receptor-γ; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Tübingen Family (TÜF) Study for type 2 diabetes. The study protocol was approved by the Ethical Committee of the University of Tübingen School of Medicine, and informed written consent had been obtained before the studies. A total of 485 subjects had NGT and 66 had IGT according to World Health Organization criteria (1). They did not take any medication known to affect glucose tolerance, insulin sensitivity, or insulin secretion. The subjects were not related. The characteristics of the subjects are shown in Table 1. The primary recruitment mechanism is by family member with type 2 diabetes. However, we included everyone in our study that was interested in a metabolic check-up (as offered by newspaper ads or fliers).

OGTT

After a 10-h overnight fast, subjects ingested a solution containing 75 g dextrose, and venous blood samples were obtained at 0, 30, 60, 90, and 120 min for determination of plasma glucose, plasma insulin, and glucagon.

Euglycemic-hyperinsulinemic and hyperglycemic clamp

In subgroups, data from euglycemic-hyperinsulinemic clamps ($n = 222$ for NGT and $n = 16$ for IGT) and hyperglycemic clamps ($n = 70$ for NGT and $n = 17$ for IGT) as previously described (7,8) were available. From the euglycemic clamp, the insulin sensitivity index (ISI), calculated as glucose infusion rate during the final 60 min divided by the mean plasma insulin concentration during this time, was used. From the hyperglycemic clamp, first-phase insulin secretion, calculated as the sum of the insulin concentrations between 2.5 and 10 min after the glucose prime, and second-phase insulin secretion, calculated as the mean insulin concentration between 80 and 120 min, were used.

Genotyping

The polymorphisms in IRS-1, IRS-2, calpain-10 (CAPN10), hepatic lipase (LIPC), and peroxisome proliferator–activated receptor-γ (PPAR-γ) were typed using standard procedures as previously described (9–13).

Definition of monophasic and biphasic plasma glucose curve shapes

The glucose curve shape of an OGTT was classified as "monophasic" when plasma glucose increased after an oral glucose load to the maximum after 30–90 min and decreased until 120 min with a final downward move of at least 0.25 mmol/l between 90 and 120 min. Glucose shapes that reached a nadir after an initial increase and increased again >0.25 mmol/l until 120 min were classified as "biphasic." The plasma glucose concentrations of a typical representative of each group (both highlighted in Fig. 1) are depicted in Fig. 2*A*. The threshold of ±0.25 mmol/l plasma glucose change was chosen empirically to avoid false classification due to experimental imprecision. In dichotomous analyses, subjects below the threshold were treated separately and referred to as "unclassified." The shape index, calculated as glucose at 90 min ($Gluc_{90}$) minus glucose at 120 min ($Gluc_{120}$) was treated as a continuous variable in correlational analyses. A shape index >0 indicates biphasic and a shape index <0 indicates monophasic. In subjects showing a decrease of plasma glucose between 30 and 60 min, an increase between 60 and 90 min, and a decrease again between 90 and 120 min (i.e., two complete peaks or a "triphasic" shape), the increase between 60 and 90 min was taken as shape index to avoid false classification of these subjects as monophasic. One IGT subject with a continuous increase during the 120 min was excluded.

Calculations and statistical analyses

Estimates for the early phase of insulin secretion and insulin sensitivity were calculated from parameters obtained during the OGTT. Estimated first-phase insulin secretion was calculated as proposed by Stumvoll et al. (2). The "insulinogenic index" was calculated as the increase in plasma insulin between 0 and 30 min divided by the glucose concentration at 30 min. An ISI was calculated as proposed by Matsuda and DeFronzo (3), and a second ISI was calculated as proposed by Stumvoll et al. (2). Homeostasis model assessment of fasting parameters for insulin resistance and insulin secretion were calculated as originally described (14). Areas under the curve (AUCs) were calculated using trapezoidal integration. A disposition index was calculated as the product of ISI (3) and estimated first phase (2).

Glucagon data were included as fasting plasma glucagon concentrations, as maximal decrease of the plasma glucagon concentrations during the OGTT, and as decrease of the plasma glucagon concentrations at 120 min during the OGTT. Unless otherwise stated, data are given as means ± SEM.

Statistical comparison of normally distributed parameters between two groups was performed using unpaired Student's *t* test. Distribution was tested for normality using the Shapiro-Wilk W test. For correlation analysis, non–normally distributed data were logarithmically transformed to approximate a linear distribution. To adjust for the effect of covariates, multivariate linear regression analyses were performed. Nominal and ordinal parameters were analyzed in a contingency table using χ^2 test. The effect of a specific polymorphism was assessed by multivariate regression analysis. A *P* value <0.05 was considered statistically significant. The statistical software package JMP (SAS Institute, Cary, NC) was used.

The statistical tests for polymor-

Table 1—*Subject characteristics*

	NGT	IGT	*P*
n	485	66	—
Sex (M/F)	188/237	24/42	0.71
Age (years)	35.4 ± 0.5	44.1 ± 1.8	<0.0001
BMI (kg/m^2)	26.1 ± 0.3	29.5 ± 0.9	<0.0001
WHR	0.85 ± 0.004	0.89 ± 0.01	0.0006
Family history of diabetes (non–first degree/first degree)	263/202	26/37	0.02
Fasting plasma glucose (mmol/l)	4.90 ± 0.02	5.59 ± 0.10	<0.0001
Fasting plasma insulin (pmol/l)	51 ± 2	80 ± 7	<0.0001
HbA_{1c} (%)	5.13 ± 0.02	5.36 ± 0.06	<0.0001

Data are means ± SEM unless otherwise indicated.

Table 2—*Subject characteristics in the monophasic and biphasic group*

	Biphasic	Unclassified	Monophasic	*P**
NGT/IGT	173/20	103/6	209/40	0.079
Sex (M/F)	60/133	32/77	120/129	0.0003
Age (years)	34.3 ± 0.8	36.3 ± 1.2	37.6 ± 0.8	0.0043
BMI (kg/m^2)	25.6 ± 0.4	25.5 ± 0.5	27.8 ± 0.4	0.0003
WHR	0.84 ± 0.01	0.84 ± 0.01	0.87 ± 0.01	<0.0001
Family history of diabetes (non–first degree/ first degree)	107/81	66/41	115/116	0.15
Fasting plasma glucose (mmol/l)	4.91 ± 0.03	4.80 ± 0.06	5.12 ± 0.04	0.0003
Plasma glucose at 120 min (mmol/l)	5.95 ± 0.12	5.42 ± 0.13	5.85 ± 0.10	0.53
$Glucose_{AUC}$ (mmol · l^{-1} · h^{-1})	12.3 ± 0.2	12.4 ± 0.2	15.1 ± 0.2	<0.0001
Fasting plasma insulin (pmol/l)	48 ± 2	49 ± 3	63 ± 4	0.0005
Plasma insulin at 120 min (pmol/l)	320 ± 20	274 ± 29	350 ± 19	0.30
$Insulin_{AUC}$ (pmol · l^{-1} · h^{-1})	620 ± 32	624 ± 44	845 ± 37	<0.0001
Fasting plasma glucagon (pg/ml)†	62.3 ± 2.4	62.0 ± 3.7	66.9 ± 2.0	0.14
Plasma glucagon at 120 min (pg/ml)†	53.5 ± 2.0	52.8 ± 3.0	54.5 ± 1.5	0.67
$Glucagon_{AUC}$ (pmol/h)†	117.8 ± 3.1	112.9 ± 4.1	117.8 ± 3.1	0.45
HbA_{1c} (%)	5.12 ± 0.03	5.10 ± 0.03	5.20 ± 0.03	0.045
ISI (ref. 3) (units)	21.5 ± 0.8	23.7 ± 1.3	16.2 ± 0.6	<0.0001
ISI (ref. 2) (units)	0.100 ± 0.002	0.101 ± 0.003	0.084 ± 0.002	<0.0001
HOMA-IR	1.74 ± 0.08	1.76 ± 0.13	2.50 ± 0.15	0.0001
Estimated first-phase insulin secretion (pmol/l)	1,148 ± 42	1,093 ± 52	1,073 ± 42	0.013
Insulinogenic index	46 ± 2	42 ± 3	40 ± 2	0.036
HOMA-IS	4,414 ± 275	3,541 ± 777	5,328 ± 326	0.13
Disposition index	21,541 ± 729	21,549 ± 984	14,021 ± 503	<0.0001

Data are means ± SEM. *χ^2 test monophasic vs. biphasic; †glucagon data were available in a subgroup of 265 subjects. HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-IS, HOMA for insulin secretion.

phisms were not corrected for multiple comparisons. A total of seven polymorphisms were examined, three of which were in the same gene. If *P* values are corrected for the fact that five different genes were examined, the relations would not remain statistically significant at the $P < 0.05$ level. However, the issue of whether it is desirable to correct for multiple comparisons has been controversial, because the overall false positive rate can only be preserved at the cost of failing to detect a true effect (15). The extent to which similar results will be shown in other studies can aid the interpretation of the plausibility of the present findings.

RESULTS — The characteristics of subjects with monophasic or biphasic shape and their OGTT data are given in Table 2. There were significantly more women than men in the biphasic group. Subjects with a biphasic shape on average had a lower age, BMI, WHR, and HbA_{1c}. They also had higher estimated insulin sensitivity and insulin secretion parameters, greater disposition index, lower fasting plasma glucose, insulin levels, and glucose and insulin AUCs in the OGTT, indicating better glucose tolerance in the biphasic group. Accordingly, there was a slightly greater proportion of IGT subjects in the monophasic group, although this did not reach statistical significance.

All of the continuous parameters, which were different between the biphasic and the monophasic groups, were significantly correlated with the shape index (Table 3, left column). The ISI from the euglycemic clamp was also positively correlated with the shape index ($r = 0.11$, $P = 0.058$). First- but not second-phase insulin secretion from the hyperglycemic clamp was correlated with the shape index ($r = 0.19$, $P = 0.08$ and $r = 0.004$, $P = 0.9$, respectively). Not surprisingly, we found a strong negative correlation of this shape index with the plasma $glucose_{AUC}$ (Fig. 1), indicating that the biphasic shape predicts good glucose tolerance. This correlation suggests that some of the differences between monophasic and biphasic subjects were simply attributable to differences in glucose tolerance.

Therefore, we adjusted the shape index for the linear relation with the plasma $glucose_{AUC}$ (Fig. 1) and repeated the above analysis using the residuals. The adjusted shape index should be independent from glucose tolerance and represents a pure measure of shape. Figure 2*A* and *B* shows the glucose and insulin curves of two representative subjects (as marked in Fig. 1) from the monophasic and biphasic groups, respectively. The subjects were selected for an identical $glucose_{AUC}$ but different shape index. In Fig. 1, the shape index is represented by the vertical distance from the dashed line (unadjusted) and from the solid regression line (adjusted).

Most of the parameters classically predicting glucose intolerance, such as old age or high BMI, were no longer correlated with the adjusted shape index. Among the continuous parameters, only fasting plasma insulin maintained a weak and not quite significant negative correlation with the shape index. The negative correlation between fasting plasma glucose and the shape index was no longer present after adjustment (Table 3, right column). None of the insulin concentration parameters was correlated with the

Table 3—*Correlations between the shape index and subject characteristics*

	Shape index		Residual shape index (adjusted for glucose$_{AUC}$)*	
	r	*P*	*r*	*P*
Age (years)	−0.170	<0.0001	0.019	0.65
BMI (kg/m^2)	−0.209	<0.0001	−0.023	0.59
WHR	−0.230	<0.0001	−0.063	0.14
Fasting plasma glucose (mmol/l)	−0.241	<0.0001	0.081	0.058
Plasma glucose at 120 min (mmol/l)	−0.066	0.12	0.397	<0.0001
Glucose$_{AUC}$ (mmol · l^{-1} · h^{-1})	−0.528	<0.0001	—	—
Fasting plasma insulin (pmol/l)	−0.250	<0.0001	−0.075	0.081
Plasma insulin at 120 min (pmol/l)	−0.119	0.0054	0.132	0.0020
Insulin$_{AUC}$ (pmol · l^{-1} · h^{-1})	−0.252	<0.0001	−0.023	0.59
Fasting plasma glucagon (pg/ml)†	−0.157	0.010	−0.009	0.88
Plasma glucagon at 120 min (pg/ml)†	−0.066	0.28	0.009	0.89
Glucagon$_{AUC}$ (pg/h)†	−0.110	0.077	0.020	0.74
HbA$_{1c}$ (%)	−0.171	<0.0001	0.006	0.89
ISI (ref. 3) (units)	0.313	<0.0001	−0.006	0.89
ISI (ref. 2) (units)	0.229	<0.0001	−0.047	0.28
HOMA-IR	−0.149	0.0005	−0.054	0.21
Estimated first-phase insulin secretion	0.128	0.0027	−0.004	0.92
Insulinogenic index	0.13	0.0025	0.040	0.35
HOMA-IS	−0.034	0.43	−0.032	0.46
Disposition index	0.464	<0.0001	−0.03	0.47

*Residuals from linear regression curve, shape-index against glucose$_{AUC}$ (for illustration see fig. 1); †glucagon data were available in a subgroup of 265 subjects. HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-IS, HOMA for insulin sensitivity.

shape index after adjusting for glucose$_{AUC}$. Similarly, neither basal glucagon concentrations nor suppression of glucagon secretion (expressed as maximal decrease during the OGTT and decrease at 120 min) had a significant effect in a multivariate regression analysis (all $P > 0.5$). The ISI from the euglycemic clamp no longer correlated with the adjusted shape index ($r = 0.009$, $P = 0.13$) and first-phase insulin secretion from the hyperglycemic clamp also no longer correlated ($r = -0.015$, $P = 0.9$).

Table 4 demonstrates the effect of adjusting the shape index in three selected subgroups. Subjects classified as IGT had a lower unadjusted but a higher adjusted shape index than subjects with NGT. Subjects classified as IGT had a lower unadjusted shape index but a higher adjusted shape index than NGT subjects. This is due to the fact that a positive shape index indicates increasing plasma glucose concentrations at the end of the OGTT, and once adjusted for glucose$_{AUC}$, a rise at the end is more likely to end up in the IGT range than a fall.

Women maintained a higher shape index also after adjustment, indicating a strong association of the biphasic shape with the female sex. This was partially and independently explained by higher early insulin secretion ($P = 0.05$), lower WHR ($P < 0.0001$), and, interestingly, lower fasting glucagon concentrations ($P = 0.0037$) in women. However, even after adjusting for these influences, women maintained a slightly but significantly ($P = 0.04$) more biphasic shape. A propensity of subjects with a family history of type 2 diabetes for any particular shape was not detected.

Among common polymorphisms in IRS-1, IRS-2, CAPN10, LIPC, and PPAR-γ, only homozygosity of the T-allele in CAPN10 UCSNP44 was associated with the monophasic shape after adjusting for glucose$_{AUC}$ and sex (Table 5).

CONCLUSIONS — In the present study, we made an attempt to extract metabolic information from the shape of the plasma glucose curve during an OGTT. Compared with the monophasic shape, subjects with the biphasic shape were characterized by younger age and a lower

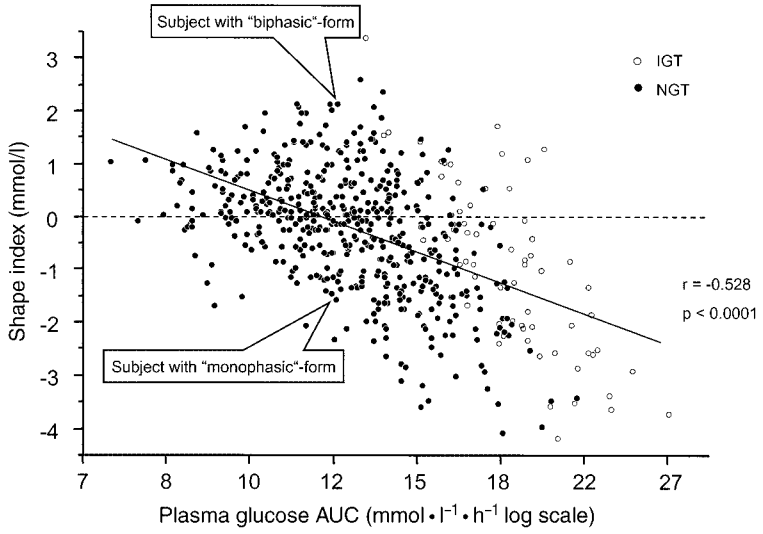

**Figure 1—*Correlation between shape index and plasma glucose$_{AUC}$. Subjects with a biphasic plasma glucose course over time in the OGTT are characterized by a shape index >0 (biphasic form), and subjects with a monophasic plasma glucose course over time in the OGTT are characterized by a shape index <0 (monophasic form). The two highlighted individuals in this figure represent NGT subjects with the same glucose$_{AUC}$ but different glucose shapes in the OGTT indicated by their difference in the shape index. The shape index was calculated as Gluc$_{90}$ − Gluc$_{120}$. A number >0 indicates biphasic and a number <0 indicates monophasic. In subjects with a decrease of plasma glucose between 30 and 60 min, an increase between 60 and 90 min, and a decrease again between 90 and 120 min (i.e., two complete peaks or a "triphasic" shape), the increase between 60 and 90 min was used as shape index. The least square regression line has the following equation: shape index = 7.73–3.13 × ln(glucose$_{AUC}$).*

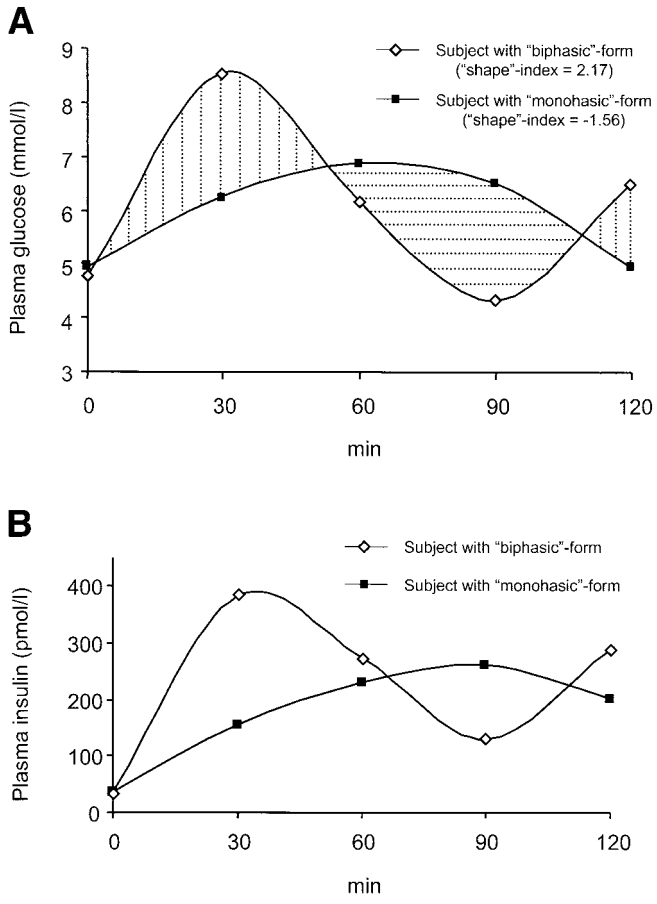

Figure 2—A: *Plasma glucose concentration during a 75-g OGTT in the individuals highlighted in Fig. 1. The vertically hatched area is identical to the horizontally hatched area.* B: *Plasma insulin concentration during a 75-g OGTT in the individuals highlighted in Fig. 1.*

BMI, WHR, fasting glucose, $glucose_{AUC}$ during the OGTT, HbA_{1c}, and insulin concentrations and higher insulin sensitivity and insulin secretion. These findings are somewhat self-fulfilling because both increased early insulin secretion and insulin sensitivity are destined to cause a more efficient disposal of the oral glucose load and result in a faster return of glucose concentrations to baseline—this is essentially what, by definition, happens in the biphasic group. We therefore developed the adjusted (for $glucose_{AUC}$) shape index, which permitted separation of the shape from the above relations. And after adjustment, the association between shape and both insulin secretion and insulin sensitivity had disappeared.

Although unadjusted, there was no significant correlation with glucose at 120 min ($glucose_{120}$), and a strong correlation appeared upon adjusting for $glucose_{AUC}$ (Table 3). It is important to understand that this does not necessarily mean that shape is simply a function of glucose tolerance. It is the result of the definition of the shape index (as difference between 90 and 120 min) that systemically "punishes" the second rise of the biphasics with a positive residual and generates a mathematical relation with $glucose_{120}$. In this context, where the whole 120 min of the OGTT is being examined, the $glucose_{AUC}$ would seem to be a more physiological measure of glucose tolerance than the classic definition, which for convenience is based on the single arbitrary time point of 120 min.

One determinant of the biphasic shape remaining after adjustment was female sex. A higher shape index in female subjects was still present after adjustment for $glucose_{AUC}$ (Table 4). Upon further analysis of our data, the preponderance of the biphasic shape in women was partially explained by higher early insulin secretion, lower WHR, and, interestingly, lower fasting glucagon concentrations. We cannot satisfactorily explain this finding, but it seems possible that subtle hormonally controlled metabolic differences that are not adequately captured by our estimates for insulin secretion or sensitivity are also involved. Nevertheless, this observation in women is reminiscent of the historical clinical diagnosis of "reactive hypoglycemia." This ill-defined term was used for a combination of postprandial symptoms resembling those of hypoglycemia and in the meantime has been abandoned (16). Notoriously, it was seen more frequently in women (17), which, despite the nosological imprecisions, is compatible with our data.

It is necessary to point out a number of limitations in our approach of assessing the shape of the glucose curve. Our definition of "biphasic" versus "monophasic" is restricted to the observational interval of 120 min. We cannot exclude that some monophasic curves had in fact a late second phase after 120 min. In any case, we

Table 4—*Differences of the shape index by sex, glucose tolerance, and family history of diabetes*

	Shape index	*P*	Residuals*	*P*
Glucose tolerance				
NGT	−0.233 ± 0.053	<0.0001	−0.055 ± 0.045	0.0009
IGT	−0.902 ± 0.200		0.403 ± 0.163	
Sex				
Male	−0.587 ± 0.087	<0.0001	−0.188 ± 0.057	0.0009
Female	−0.141 ± 0.065		0.118 ± 0.070	
Family history of diabetes				
non–first degree	−0.206 ± 0.070	0.12	−0.035 ± 0.061	0.16
first degree	−0.372 ± 0.083		0.094 ± 0.070	

Data are means ± SEM. *Residuals from linear regression curve, shape index against $glucose_{AUC}$ (for illustration see fig. 1).

Table 5—*Shape index in selected genotype groups*

Polymorphism		Shape index	*P*	*P* adjusted*
LIPC-514C/T	CC	−0.294 ± 0.064	0.86	0.81
	TC + TT	−0.330 ± 0.094		
CAPN10 UCSNP43	GG + GA	−0.270 ± 0.039	0.16	0.17
	AA	−0.526 ± 0.174		
CAPN10 UCSNP44	CC + CT	−0.110 ± 0.092	0.082	0.011
	TT	−0.365 ± 0.068		
CAPN10 UCSNP45	AA	−0.259 ± 0.062	0.45	0.21
	CA	−0.465 ± 0.195		
IRS-1 Gly972Arg	Gly/Gly	−0.294 ± 0.060	0.77	0.76
	Gly/Arg + Arg/Arg	−0.252 ± 0.143		
IRS-2 Gly1057Asp	Gly/Gly	−0.375 ± 0.083	0.099	0.33
	Gly/Asp + Asp/Asp	−0.227 ± 0.074		
IRS-2 Gly1057Asp	Gly/Gly	−0.375 ± 0.083	0.096	0.084
	Asp/Asp	0.081 ± 0.143		
PPAR-γ Pro12Ala	Pro/Pro	−0.303 ± 0.062	0.94	0.98
	Pro/Ala + Ala/Ala	−0.292 ± 0.104		

Data are means ± SEM. The subjects are the same as in table 3; **P* for residuals from a standard least square model taking into account sex and $glucose_{AUC}$.

classified those who completed the second phase within 120 min, which by itself is probably already meaningful, as biphasic. Similarly, finer oscillations may have been missed due to the sampling width of 30 min. This makes it also difficult to apply mathematical modeling to the datapoints, a theoretically useful approach to quantify phasicity. Our "shape" index may not be appropriate for every conceivable glucose curve, but it is simple to use and requires very few measurements. (Adjustments for $glucose_{AUC}$ can be done using the equation given in the legend of Fig. 1.)

What are the physiological processes contributing to the shape of the glucose curve? Based on the unadjusted shape index, both insulin sensitivity and insulin secretion are obviously involved. Once adjusted for $glucose_{AUC}$, however, the two crude measurements of these processes were no longer significant, so others must be considered. Clearly, gastric emptying plays an important role and could explain the slope of the initial rise. We have no information on this variable and it is possible that the reoccurrence of the rise in the biphasic group can be explained with variability in the rate of gastric emptying. However, classic double isotope studies failed to detect differences in magnitude or time course of systemic appearance of an oral glucose load between healthy and type 2 diabetic patients (18). The insulin concentrations (Fig. 2*B*) have a shape very similar to that of the glucose curve. It appears that they are a consequence rather than a cause of the glucose concentrations, although this is ultimately difficult to assess. Glucose concentrations during an OGTT are not only determined by insulin-stimulated glucose disposal, but also by the suppression of endogenous glucose production. It is therefore possible that hepatic insulin sensitivity also contributes to the individual shape of the glucose curve.

Furthermore, glucagon secretion is suppressed during an OGTT and inadequate suppression contributes to IGT (19). The suppressibility of glucagon secretion did not seem to play a role on the shape itself. However, we cannot exclude that, in addition to hepatic insulin sensitivity, hepatic glucagon sensitivity is involved. In summary, we are unable to provide a comprehensive picture as to what determines the individual glucose shape. Nevertheless, we propose that, considering the widespread availability of glucose tolerance measurements, the shape index (or improved measures of glucose shape) may turn out to be a useful screening parameter for specific abnormalities in hepatic insulin and glucagon action in epidemiological or genetic studies.

Finally, we applied the shape index (adjusted and unadjusted) to genetic association studies. We tested whether common polymorphisms previously reported to be associated with type 2 diabetes or related disorders had an independent effect on the shape of plasma glucose during an OGTT. We realize that testing associations with polymorphisms may seem circular, since we cannot yet provide a complete understanding of the metabolic components underlying the adjusted shape. However, it is possible that the shape harbors metabolic subtleties, such as hepatic glucagon or glucose sensitivity, for example, which may be modulated by genetic variants. Therefore, we would like to explicitly point out the preliminary and hypothesis-generating nature of our genetic association data.

The Gly972Arg polymorphism in IRS-1 was found to be associated with reduced insulin secretion in some (10,20) but not all studies (21). The Gly1057Asp polymorphism in IRS-2, except for an interaction with obesity on diabetes risk, was not found to be of metabolic relevance (9,20). The uncoding single neucleotide polymorphisms in the CAPN10 gene (UCSNP43, UCSNP44, and UCSNP45) were reported to have some association with metabolic traits by some (22–25) but not all authors (11). The −514 C/T polymorphism in the LIPC gene was reported to influence plasma VLDL concentrations (13) but has not been assessed for other diabetes-relevant traits. The Pro12Ala polymorphism in the PPAR-γ_2 gene is associated with reduced risk of type 2 diabetes and increased insulin sensitivity (26,27). The relation with insulin secretion is unclear (28–30). We found an association of the T-allele in CAPN10 UCSNP44 with the monophasic shape. This relation became significant using the adjusted shape index. Moreover, the Asp allele in IRS-2 appeared to be associated with the biphasic shape, but this failed to remain significant after adjusting for $glucose_{AUC}$.

In conclusion, the shape of the plasma glucose curve during an OGTT can be easily assessed and may contain the net information of a number of metabolic factors, including insulin sensitivity, insulin secretion, glucagon secretion and sensitivity, and hepatic glucose sensitivity. The biphasic shape is associated with NGT and female sex. Among common genetic variants implicated in metabolic disorders, the UCSNP44 C/T polymorphism in CAPN10 had a significant effect on the adjusted shape index. Although the physiological factors influencing the glucose

shape during an OGTT remain to be determined, the shape index may be a useful metabolic screening parameter in epidemiological and, perhaps, genetic association studies.

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