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RESEARCH ARTICLE

Glucose time series complexity as a predictor of type 2 diabetes

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Abstract

Background Complexity analysis of glucose profile may provide valuable information about the gluco-regulatory system. We hypothesized that a complexity metric (detrended fluctuation analysis, DFA) may have a prognostic value for the development of type 2 diabetes in patients at risk.

Methods A total of 206 patients with any of the following risk factors (1) essential hypertension, (2) obesity or (3) a first-degree relative with a diagnosis of diabetes were included in a survival analysis study for a diagnosis of new onset type 2 diabetes. At inclusion, a glucometry by means of a Continuous Glucose Monitoring System was performed, and DFA was calculated for a 24-h glucose time series. Patients were then followed up every 6 months, controlling for the development of diabetes.

Results In a median follow-up of 18 months, there were 18 new cases of diabetes (58.5 cases/1000 patient-years). DFA was a significant predictor for the development of diabetes, with ten events in the highest quartile *versus* one in the lowest (log-rank test chi2 = 9, df = 1, $p = 0.003$), even after adjusting for other relevant clinical and biochemical variables. In a Cox model, the risk of diabetes development increased 2.8 times for every 0.1 DFA units. In a multivariate analysis, only fasting glucose, HbA_{1c} and DFA emerged as significant factors.

Conclusions Detrended fluctuation analysis significantly performed as a harbinger of type 2 diabetes development in a high-risk population. Complexity analysis may help in targeting patients who could be candidates for intensified treatment.

Keywords detrended fluctuation analysis; complexity; type 2 diabetes; continuous glucose monitoring

Introduction

While diagnostic criteria for type 2 diabetes are well established [1], it is generally admitted that when these criteria are met, significant damage has already been done, and at diabetes diagnosis, pancreatic beta function has lost 50% of its capacity [2,3]. The fasting plasma glucose 126 mg/dL and glycosylated haemoglobin (HbA_{1c}) 6.5% threshold were established based on the risk of developing diabetic retinopathy, but insulin secretion and endothelium are

known to be at risk long before this threshold is reached [4]. Of course, changing the threshold is not the answer: it would only increase the rate of false positives.

Furthermore, although two of the conventional criteria, HbA_{1c} and oral glucose tolerance test, somehow contemplate the temporal dimension of the problem, the diagnosis of diabetes is mostly a categorical 'yes/no', snapshot-decision that overlooks the essentially dynamic aspect of the question, in two timescales:

- in the long term (months–years): diabetes is the end of a process that often begins with some very prevalent conditions (e.g. being overweight).
- in the short term (minutes–hours): arguably, the first disturbance in gluco-regulation is the disruption in the physiologic flux and reflux of glycolysis and gluconeogenesis that occur in the feeding and fasting cycle, appearing first either as impaired fasting glucose or as impaired glucose tolerance.

It would be of great interest to find an instrument that could explore the short-term glucose dynamics and perhaps provide a tool to follow quantitatively the evolution from prediabetic conditions like the metabolic syndrome and other phenotypes of increased diabetes risk to type 2 diabetes meeting current standard diagnostic criteria.

This has been tried through conventional variability metrics for glucose time series, mostly derived from range or standard deviation (SD). However, these approaches have significant limitations, most importantly the fact that they assume each measurement as an independent value, which it obviously is not: the present level of glycaemia is heavily conditioned by the previous. A consequence of this limitation is the fact that the same set of glucose measurements analysed in an orderly fashion or in a random order will have the same (conventional) distribution, but obviously entirely different biological meaning because it does not take into account an essential characteristic of a time series, namely, its sequentially. There have been attempts to consider this aspect, mainly by means of the mean amplitude of glycaemic excursions (MAGE) [5], but this metric is hampered by an arbitrary definition of glycaemic excursion (i.e. one SD of the time series under analysis) and has not found generalized acceptance in diabetes.

Complexity analysis of time series is a set of techniques derived from non-linear dynamics that may provide a useful insight into this issue. A full discussion on complexity analysis falls out of the scope of the present article, but in essence, these methods explore the informational content ($\approx$ entropy) of a time series analysing the rate at which details are lost as the time window increases (and thus the description becomes less meticulous). A more specific description of detrended fluctuation analysis (DFA; the tool used in this study) is offered in the Methods section, and a brief elementary description can be found in http://www.complexity-at-the-bedside.org/complexity/tutorials/. The main idea is that the more complex a time series, the more its description will depend on the small details. If one builds a 'map' of a time series through a set of linear segments of varying sizes (time windows) and measures the gap between the 'map' (the linear segments) and the 'territory' (the time series) (Figure 1), it is apparent that the larger the time windows, the larger the 'map-to-territory gap'. DFA measures the rate at which this gap increases as the time windows enlarge. A more complex series will have proportionally more of its information codified in the small windows, so a sizable rise in the map-to-territory gap will occur in the small windows. Conversely, a less complex series will have more information codified in the large windows, and therefore the map-to-territory gap will increase steadily well into larger time windows. This is displayed as a higher DFA (i.e. DFA increases as the complexity decreases).

An almost ubiquitous finding in complex systems is the 'de-complexification' of their output as the system decays. This has been observed in heart rhythm, thermoregulation, ageing or neurologic disorders [6–10], among others, and it is often one of the earliest signs of disease.

Gluco-regulation is a paradigmatic example of a complex system, with several overlapping feedback and feed forward loops, and therefore it would be reasonable to expect a loss of complexity in its output as an early symptom of dysfunction. In fact, several publications have reported such a loss both in type 1 diabetes and type 2 diabetes, and there seems to be a relation between loss of complexity and progression in dysglycaemia [11–18].

Our present study examines whether complexity analysis of glucose time series may provide more information, over and above that supplied by conventional variables (fasting glucose, HbA_{1c}, etc.), on the decay of gluco-regulation and namely if it can help in predicting which patients at risk will eventually run the whole way to diabetes. We present a prospective survival analysis of the incidence of diabetes in a population with high risk of developing this disease.

Material and methods

Patients

The study targeted at patients considered at increased risk of developing diabetes. This was defined as complying with at least one of the following conditions:

a essential hypertension,
b BMI $\geq$ 30 kg/m^2,
c a first-degree relative diagnosed of diabetes.

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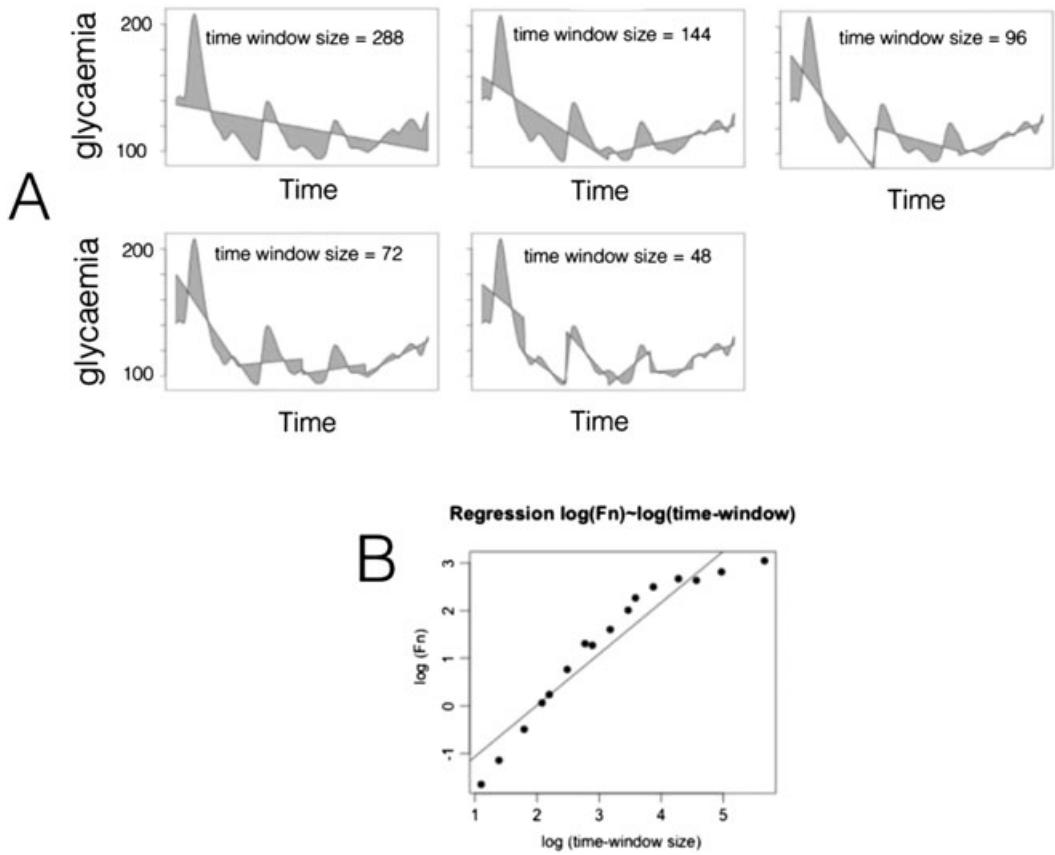

Figure 1. The gap between the regression line(s) and the glucose curve is calculated for different time-window sizes:

$$Fn = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[y(k) - y_n(k)]^2},$$

where N is the number of point in the time series, $y(k)$ is the value of the time series at time k and $y_n(k)$ is the value of the linear regression at time k. In our series, $N = 288$, corresponding to a glucose measurement every 5 min for 24 h. The time windows used went from one 24-h time window (288 points) to ninety-six 15′ windows (three points in each window). (A) It displays *Fn* for time-windows size of 288 points (one 24-h window), 144 (two 12-h window), 96 (8 h), 72 (6 h) and 48 (4 h). *Fn* is calculated for progressively smaller time windows, to a limit of 15 min. (B) If the series has a fractal structure, a regression model can be built for log(*Fn*) ~ log(time-window size). Detrended fluctuation analysis is the slope of this regression line

A total of 262 patients were finally recruited from the General Internal Medicine outpatient clinic and the Hypertension Unit of the Mostoles University Hospital from January 2012 to December 2014.

Patients under 25 or over 85 years old were excluded, as were those with a previous diagnosis of diabetes, taking anti-diabetic drugs or on treatments that could affect gluco-regulation (e.g. glucocorticoids).

At admission, patients underwent a general history, physical exam and routine biochemical tests (including fasting glucose, lipid profile, HbA_{1c}, fasting insulin, homeostasis model assessment-insulin resistance index, serum cystatin C and albuminuria).

Then a glucometry was performed by means of a continuous glucose monitoring system. After that, patients were reviewed every 6 months (general examination and biochemical tests) until the end of the study.

An event (diagnosis of diabetes) was considered when a patient had either the following:

- fasting glucose $\geq$ 126 mg/dL,
- $HbA_{1c} \geq 6.5\%$,
- started on anti-diabetic drugs.

When the results of two different tests (e.g. fasting glucose and HbA_{1c}), available for the same patient, resulted both above the diagnostic thresholds, the diagnosis of diabetes was made. Otherwise, the diagnosis was confirmed on a second test.

The study protocol was approved by the Hospital's Ethical Committee, and a written informed consent was obtained from each patient.

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Glucometry

At admission to the study, every patient underwent a glucometry (iPro; Medtronics MiniMed, Northridge, CA, USA) for 3 days, while the patient followed his normal life (including normal diet). This technique allows determining glucose in interstitial fluid every 5′. Once downloaded, the time series was revised, and a clean 24-h segment was selected for analysis. Whenever possible, the selected 24 h started at 08.00 h on day 2 (to avoid the stressful hours in hospital). However, complexity analysis ideally requires a complete time series, with no interruptions, which occasionally is not possible. If the time series had missing values, they were calculated by interpolation, as long as the missing string was <3 consecutive values. If there were three or more consecutive missing values, the time series was considered inadequate, and another 24-h period was selected from the same glucometry. If no adequate 24-h period was found, the glucometry was considered unsuitable and was discarded.

Complexity analysis

Complexity analysis was performed by means of DFA. A full description of DFA can be found in [19], and a basic introductory video can be seen at http://www.complexity-at-the-bedside.org/complexity/tutorials/. Basically, DFA explores the complexity of a time series analysing the rate of information loss as the 'graining' of the description becomes coarser. The central idea is that complex series will have a greater amount of information in the small details, and consequently, their description will become rapidly more and more inaccurate as the graining (time-window size) increases, while less complex time series will retain a more accurate description well into coarser graining. The algorithm proceeds as follows:

1 The series is divided into W equally distributed time windows, with a window size.

$$n = \text{total number of lectures} / W$$

2 A linear regression is calculated for each window.
3 The 'error' (area between the curve and the linear regression) is measured for the whole series.

Formally,

$$Fn = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[y(k) - y_n(k)]^2},$$

where Fn is the measure of the difference between the curve and the regression line, N is the total number of data points, $y(k)$ is the value of the curve at each point and $y_n(k)$ is the value of the regression line at that point.

This area (Fn) can be interpreted as the gap between the 'territory' (the time series) and the 'map' (the linear regression).

4. The process is repeated for a set of different time windows, and Fn is found for each n (Figure 1A).
5. In complex systems deploying fractal characteristics, there is an exponential relation between Fn and the time-window size (Fn n^a). Consequently, there will be a linear relation between $\log(Fn)$ and $\log(n)$.

DFA (a) is the slope of the relation: $\log(Fn)$ $a \cdot \log(n)$ (Figure 1B).

Complex time series will quickly increase their 'map *versus* territory gap' as the 'graining' (time-window size) increases, therefore displaying proportionally higher Fn values in small windows, while less complex series will keep this gap proportionally small until larger time-window sizes. Consequently, complex series will have a less steep $\log(Fn)$ $\log(n)$ slope and a lower DFA (Figure 1B).

It should be noted that, while most articles applying DFA to biological signals integrate the time series before the detrending, (including our previous articles) [11,17,18], we now omit this pretreatment. Integrating the time series is used in long, noisy, non-stationary time series, converting them into a random-walk model, and thus allowing the use of all the mathematical tools developed for this model. Most notably, this establishes the threshold or DFA = 1.5, for a random series. Therefore, DFA > 1.5 denotes a positive correlation, while DFA < 1.5 indicates an anti-correlated time series. However, our time series is short (288 points) and fairly stationary, and 'smoothing' them through integration arguably erases valuable information. Admittedly, omitting the initial integration precludes the conventional 'random-walk' interpretation of DFA. However, this metric remains a solid probe to explore the 'map–territory gap' enlargement as the time windows increase and thus provides a useful measure of the time series' entropy.

Both complexity analysis and statistical analysis were performed in R (http://cran.r-project.org/). Normality was analysed by means of the Shapiro–Wilk test. Principal component analysis was calculated by means of the correlation matrix (principal {psyche} cran.r-project.org/web/packages/psych/psych.pdf), with factors selected according to the Kaiser–Meyer–Olkin criterion (eigenvalue > 1).

Significance was defined by a two-tailed $p < 0.05$.

Results

Initially, 262 patients were recruited. From this cohort, 40 were excluded because their glucometries were

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considered unsuitable for complexity analysis. A total of 15 patients had no follow-up, and one patient was excluded because she started on high-dose corticosteroids because of a facial palsy. The remaining 206 patients are the object of the present analysis.

Except for a slightly lower diastolic blood pressure (73.9 mmHg *versus* 78.1 mmHg, $p = 0.01$) and a tendency towards higher triglycerides levels (110 mg/dL *versus* 123 mg/dL, $p = 0.09$), there were no significant differences between included and excluded patients regarding gender, age, body mass index, abdominal circumference, systolic blood pressures, fasting glucose, HbA_{1c}, high-density lipoprotein (HDL)-cholesterol, renal function, albuminuria or number of Adult Treatment Panel-III metabolic syndrome defining criteria (data not shown). Therefore, exclusion does not seem to carry any bias.

The principal clinical characteristics of the finally included population are shown in Table 1.

Table 1. Clinical variables of study population at entry

	All: 206
Age (years)	
Median (IQR)	61 (13)
Gender	
Female/male	101/105
Relatives with diabetes (%)	55 (28)
Obesity (BMI ≥ 30) (%)	95 (46)
Essential hypertension (%)	189 (92)
Systolic BP (mmHg)	
Median (IQR)	133.5 (19.25)
Diastolic BP (mmHg)	
Mean (SD)	78.2 (9.0)
BMI (Kg/m^2)	
Median (IQR)	30 (6)
Abdominal circumference (cm)	
Men	
Mean (SD)	104.5 (10.1)
Women	
Mean (SD)	99.2 (12.1)
Fasting glucose (mg/dL)	
Mean (SD)	100.18 (11.17)
HbA_{1c} (%)	
Median (IQR)	5.8 (0.29)
IFG (%)	105 (51%)
$HbA_{1c} \geq 5.7$ (%)	129 (66%)
HDL-cholesterol (mg/dL)	
Men	
Median (IQR)	43.8 (13.5)
Women	
Median (IQR)	57.9 (12.3)
Triglycerides (mg/dL)	
Median (IQR)	110 (62.8)
EPI-GFR (mL/min/1.73 m2)	
Mean (SD)	93.0 (9.5)
Insulin (pmol/L)	
Median (IQR)	70.2 (57)
HOMA-index	
Median (IQR)	3.06 (2.27)
Albuminuria (mg/g creatinine)	
Median (IQR)	2.78 (6.15)
Number of ATP-III MS defining criteria	
Median (IQR)	2 (1)
Number of patients complying with the ATP-III MS definition (≥3 criteria)	100 (49%)
Smoking habit (%)	23 (11%)
CV (%) glucose time series	
Median (IQR)	14.2 (6.7)
MAGE (mg/dL)	
Median (IQR)	2.02 (1,27)
DFA	
Mean (SD)	0.90 (0.09)

BP, blood pressure; BMI, body mass index; IFG, impaired fasting glucose (fasting glucose ≥ 100 mg/dL); EPI-GFR, estimated glomerular filtration rate (EPI-creatinine equation); HOMA, homeostasis model assessment; MS, metabolic syndrome; CV, coefficient variation; MAGE, mean average glucose excursions; DFA, detrended fluctuation analysis; IQR, interquartile range; SD, standard deviation; ATP, Adult Treatment Panel.

Correlations within variables at entry

Complexity showed a significant negative correlation (positive correlation with DFA) with several variables known to be risk factors for the development of diabetes, namely, the abdominal circumference, fasting glucose, HbA_{1c}, the number of metabolic syndrome-defining criteria and MAGE (Table 2). There was also a (paradoxical) negative correlation between diastolic blood pressure and DFA, but it disappeared when adjusted by the variable of being on antihypertensive drugs treatment (either as a qualitative variable or as the number of antihypertensive drugs taken by the patient).

Events

During a median follow-up of 17.5 months, 18 events were recorded: four (22%) because of fasting glucose ≥126 mg/dL, three (17%) because of $HbA_{1c} \geq 6.5\%$, ten (56%) because of both criteria and one patient because she was started (in another centre) on anti-diabetic drugs. This represents an event-ratio of 58.25 cases/1000 patient-years.

Survival analysis

A univariate Cox proportional hazard model for diabetes development was built for different variables (Table 3).

In addition to several conventional variables (abdominal circumference, fasting glucose, HbA_{1c}, HDL-cholesterol, number of Adult Treatment Panel-III metabolic-syndrome defining criteria and MAGE), DFA had a significant influence on the hazard ratio of diabetes development (beta = 11.434). This implies that the odds-ratio of developing diabetes increases 3.14 times for each 0.1 unit of increase in DFA.

In the log rank test, there was one event in the lowest DFA quartile *versus* ten in the highest (chi-square 9 (df = 1), $p = 0.003$).

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Table 2. DFA: correlations with clinical variables (with statistical signification)

	Correlation [a]	*p*
Abdominal circumference (cm)	0.144	0.04
Fasting glucose (mg/dL)	0.153	0.03
HbA_{1c} (%)	0.290	<0.001
Number of MS defining criteria	0.161 [b]	0.02
CV glucose time series	0.62 [b]	<0.001
MAGE (mg/dL)	0.746 [b]	<0.001
Diastolic blood pressure	−0.165	0.02

MS, metabolic syndrome; CV, coefficient variation; MAGE, mean average glucose excursions; DFA, detrended fluctuation analysis.
[a]Pearson's *r*, unless stated otherwise.
[b]Spearman's rho.

Table 3. Cox survival univariate analysis

Independent variable [a]	Coefficient	Effect	*p*
DBP	0.057	1.059	0.04
Fasting glucose	0.160	1.174	<0.001
HbA_{1c}	5.755	316	<0.001
MS	2.300	9.965	0.002
MS-glucose criteria	2.436	11.433	0.001
MS-HDL-Chol- criteria	1.518	4.564	0.002
MS-triglycerides-criteria	2.101	8.172	<0.001
MS-number criteria	1.091	2.977	<0.001
IFG	2.174	8.796	0.004
MAGE	0.0194	1.0196	<0.001
DFA	11.434	92375	<0.001

Dependent variable: development of diabetes.
DBP, diastolic blood pressure; MS, metabolic syndrome; IFG, impaired fasting glucose (fasting glucose ≥ 100 mg/dL); MAGE, mean average glucose excursions; DFA, detrended fluctuation analysis; HDL, high-density lipoprotein.
[a]At entry. Only variables with statistical signification are shown.

Multivariate models

A multivariate Cox survival model for diabetes was built, including as independent variables, MAGE, DFA and all clinically relevant variables (fasting glucose, HbA_{1c}, age, gender, relatives with diabetes diagnosis, smoking habit, body mass index, abdominal circumference, systolic blood pressure, HDL-cholesterol and triglycerides). In such a model, only fasting glucose HbA_{1c} and DFA emerged as significant (Table 4).

Principal component analysis

We were interested in studying how the variables strictly related with glucose levels (fasting glucose and HbA_{1c}) and the variables related with glucose dynamics (MAGE and DFA) interacted in the evolution to diabetes. To do so, we tried building a multivariate Cox proportional hazard model including all these variables as independent factors. However, because of the high degree of multicollinearity, all four variables and each and every one of their interactions were significant (data not shown), giving rise to results that were uninterpretable. To face this problem, we performed a principal component analysis with varimax rotation. Following the Kaiser–Meyer–Olkin criterion (eigenvalue > 1), two principal components were selected, capturing 0.75 of the total variance, and quite adequately representing the variables related with glucose levels (*RC*2, 'glycaemia variables') and glucose dynamics (*RC*1, 'dynamic variables') (Figure 2). Loadings of principal components analysis were as follows: for DFA, $RC1 = 0.90$ and $RC2 = 0.15$; for MAGE, $RC1 = 0.90$ and $RC2 = 0.09$; for basal glycaemia, $RC1 = 0.03$ and $RC2 = 0.84$; and for HbA_{1c}, $RC1 = 0.18$ and $RC2 = 0.78$.

Table 4. Cox survival analysis, including DFA and all clinically relevant variables

	Beta	Effect	*p*
Fasting glucose	0.0958	1.101	0.005
HbA_{1c}	4.342	7.683	0.005
DFA	8.607	5.472	0.008

The rest of included variables (age, gender, relatives with a diabetes diagnosis, smoking habit, body mass index, abdominal circumference, systolic blood pressure, HDL-cholesterol and triglycerides) resulted excluded in the final model.
DFA, detrended fluctuation analysis.

In a Cox survival analysis, both factors were significant: for *RC*1 ('dynamic variables'), beta = 0.972 (effect = 2.646, $p < 0.0001$) and for *RC*2 ('glycaemic variables'), beta = 2.023 (effect = 7.565, $p < 0.001$).

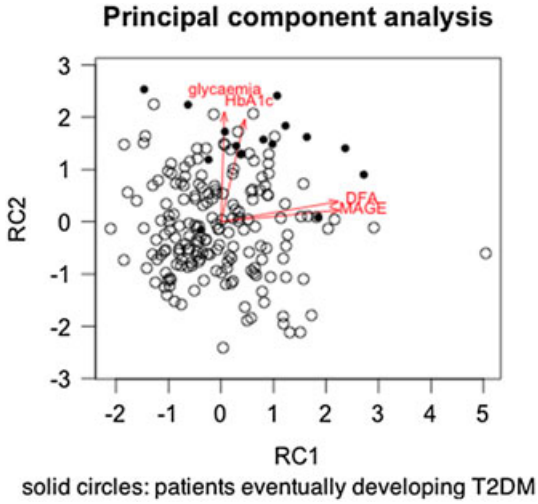

Figure 2. Principal component analysis: biplot representation. *RC*1: DFA and MAGE (glucose dynamics components). *RC*2: fasting glucose and HbA_{1c} (glucose level components). DFA, detrended fluctuation analysis; MAGE, mean average glucose excursions; T2DM, type 2 diabetes mellitus

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Discussion

In our population, the complexity of glucose time series, measured by DFA, was an independent predictor of diabetes development. In a univariate analysis, the probability of diabetes development increased more than three times for each 0.1 unit of increase in DFA (lesser complexity), so that there were ten events in the highest DFA quartile versus one event in the lowest.

Both variables measuring the 'dynamical characteristics' of the time series (DFA and MAGE) were independent predictors of diabetes development; however, in a head-to-head bivariate comparison, only DFA remained significant, suggesting a better discriminant capacity.

While at first glance the inverse correlation between complexity and variability (i.e. direct correlation between DFA and MAGE) may seem counter-intuitive, it is just what one would expect. A healthy regulatory system should be able to detect and correct minor departures from 'normality', displaying a 'ragged' output, with numerous small 'ups-and-downs' (high complexity). A failing system would show a decreased sensitivity and/or a slower and/or less efficient response, allowing for larger oscillations (higher variability).

Detrended fluctuation analysis remains as a significant predictor of diabetes development in a multivariate model including all the other clinical variables. Furthermore, by means of principal component analysis, we were able to separate the factors mainly related to glucose levels (fasting glucose and HbA_{1c}) from those related to glucose dynamics (DFA and MAGE), and both proved to have significant influence on diabetes development. The 'glycaemic factors' had a stronger weight in the model than the 'dynamic' elements, but this may be heavily influenced by the fact that 'glycaemic variables' are precisely the (future) event-defining items.

As we have commented previously, we omit in the present analysis the integration of the glucose time series before detrending. However, we performed the same statistical analysis with this preprocessing and obtained very similar results (integrated DFA was a significant predictor of diabetes it correlated with MAGE, etc.), but the predictive power (beta) was significantly smaller. Therefore, we decided to omit the initial integration, and use DFA as a blind metric, without assuming the conceptual background supplied by the random-walk model. This explains the considerably lower DFA values of our series (mean 0.899, SD 0.087). When integrated, our results are similar to other studies (mean 1.415, SD 0.093).

Several authors have emphasized the influence of dynamic aspects of glycaemia on the development of diabetes and its complications, not just because of the risk of hypoglycaemia but mainly through the oxidative stress induced by acute glucose swings [20–24]. However, this hypothesis is not uniformly accepted [25], and the question of which metrics should be used is still under debate [26].

In a previous cross-sectional study [11], we observed that the complexity of glycaemic profile decreased from healthy individuals, through the metabolic syndrome, to early diabetes. In the present survival analysis, we tease-out the influence of glucose levels from that of glucose dynamics, proving their relative influence on the development of diabetes, thus supporting the importance of variability and complexity in the study of glucose dysregulation.

In the last years, continuous glucose monitoring system is becoming a common tool in type 2 diabetes [12–16]. Kohnert K-D *et al*. [13] described that, in patients with diabetes without anti-diabetic drug treatment, decreasing complexity and increasing variability are associated with declining beta-cell reserve and worsening glycaemic control. Ogata *et al*. [12] reported significant correlations between HbA_{1c}, glycated albumin and the long-range scaling DFA exponent, suggesting that an increase in this parameter reflects clinically relevant abnormalities in average glycaemic control. Costa *et al*. [16] performed continuous glucose monitoring (CGM) in a group of elderly subjects and concluded that the dynamics of glucose fluctuations from healthy subjects are more complex than those from patients with diabetes over time scales ranging from about 5 min to 5 h. Chen *et al*. [27] compared the complexity of glucose dynamics in patients with diabetes (including type 1 and type 2 diabetes) with controls and also reported a decreased complexity (assessed by multiscale entropy analysis) in patients with type 2 diabetes.

Continuous glucose monitoring system technology is also being increasingly used in prediabetic conditions, mainly to assess glycaemic variability [28–33]: Chen *et al*. [31] and Wang *et al*. [32] report a progressive increase in 24-h mean basal glucose and MAGE from normal glucose regulation, throughout impaired glucose tolerance to diabetes. In both studies, the diagnosis was made by means of 2-h oral glucose tolerance test. However, these studies use conventional statistics such as SD, coefficient of variability or MAGE, which we would suggest are not as sensitive or robust as complexity metrics. Yamamoto *et al*. [33] classified in these same three categories of glucose derangement according to CGM data (which is not a fully validated clinical method) and found a progressive loss of complexity (measured by DFA) in the glycaemic profile with the progression from normal glucose metabolism to the diabetes state.

To the best of our knowledge, this is the first prospective analysis demonstrating the influence of glucose complexity on the development of diabetes. The incidence of diabetes in our sample (58.25 cases/1000 person-years) is comparable with that reported in similar cohorts [34–36], and

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therefore our methodology and conclusions may be extensible to other populations.

Now that new treatments to delay or prevent the progression of prediabetes to diabetes are available [37–41], screening high-risk patients for early diagnosis and management of glycaemic abnormalities becomes ever more important.

In our opinion, the present findings raise the question of whether complexity analysis of glucose–time series obtained by CGM in prediabetic patients can help in the risk assessment of progression to diabetes.

Our study has several limitations. We could not compare the prognostic value of DFA with HbA_{1c} and basal glucose for the risk assessment of cardiovascular complications because of the small number of these events during the follow-up. Another limitation was the lack of a basal oral glucose tolerance test for a better classification of the glucose metabolism disruption (e.g. impaired glucose tolerance). However, no patient had diabetes (defined as any postprandial glucose ≥200 mg/dL during the whole register) on the CGM time series.

In conclusion, in our population, complexity of glycaemic profile measured by DFA was an independent predictor of diabetes development. Performing a CGM that includes DFA assessment could be a useful tool in diabetes-risk evaluation in this kind of patients.

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