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ORIGINAL ARTICLE

Methodology for Quantifying Fasting Glucose Homeostasis in Type 2 Diabetes: Observed Variability and Lability

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Abstract

Background:

Increased glycemic variability is associated with an increase risk of adverse clinical outcomes in diabetes. Central to the understanding of diabetes is glucose homeostasis. "Good" homeostasis is equated to low glycemic variability, and "poor" homeostasis is linked to greater glycemic variability. We have, therefore, developed a method with the aim to objectively quantify the domain of glucose–insulin homeostasis. We have termed this method as Observed Variability And Lability (OVAL).

Method:

Blood samples for the measurement of glucose and insulin concentrations were acquired every 2 min for 120 min from 12 patients with type 2 diabetes mellitus [T2DM; median (range) age 35 (25–47) years and duration of diabetes 7 (2–9) years receiving oral hypoglycemic treatment] and 27 controls [aged 38(30–53) years] with an equal split of genders and equal distribution of body mass indexes. The insulin–glucose time variant data form the boundaries of OVAL, defined as the ellipse enclosing the 95% confidence intervals of the insulin and glucose concentrations plotted on an x–y scatter graph and normalized to ensure equal weighting of insulin and glucose.

Results:

Less precise OVAL homeostasis was observed in subjects with T2DM, by a factor of 4, in comparison with controls [OVAL, T2DM 7.8(3.8) versus controls 1.9(1.0); $p = .0003$]. The assessment remained statistically robust ($p < .001$) with increased sampling intervals up to 8 min.

Conclusion:

The OVAL model is a robust method for measuring glucose–insulin homeostasis in controls and T2DM subjects (available online at http://www.oval-calc.co.uk). Deranged glucose–insulin homeostasis is the hallmark of diabetes and OVAL has the capacity to quantify in the fasting state.

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Abbreviations: (CI) confidence interval, (HOMA) homeostatic model assessment, (OVAL) observed variability and lability, (SD) standard deviation, (T2DM) type 2 diabetes mellitus

Keywords: glucose homeostasis, glucose insulin homeostasis, glucose variability, glycemic lability

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Introduction

When evaluated, the properties of many biological processes, such as hormonal secretion, demonstrate a feedback control system—referred to in biology as "homeostasis." However, within the homeostatic boundaries of a system, the behavior appears neither predictable nor repeatable. The traditional view of biological systems like these is that they are complex with a large number of degrees of freedom that are not directly observed but nevertheless exert an effect on the process.[1]

Glucose homeostasis *in vivo* can be represented as the steady-state basal concentration of glucose that is maintained by the feedback processes of insulin and other hormones.[2] The concept of the stable biological internal environment was first described in the 1850s by Claude Bernard,[3] and the term homeostasis was first coined in 1926 by Walter Cannon.[4] In basal conditions, glucose homeostasis is maintained by glucose-dependent insulin release. In those with diabetes, the glucose-dependent response is dependent on the nature of the pathology, which is variable and can be predominantly loss of beta-cell function and/or an increase in insulin resistance or a combination of both these states.[5]

Glucose homeostasis is an important aspect in the regulation of blood glucose concentrations, and understanding this system is central to understanding diabetes. Homeostasis alteration in the fasting state has been examined in greater than 150 epidemiological studies using the homeostatic model assessment (HOMA) model, and it has been recognized that diabetes affects ethnic groups differently through altered beta-cell dysfunction and abnormal insulin resistance.[6] The study of glucose homeostasis has helped in clarifying the pathology of genetic subgroups of diabetes such as maturity-onset diabetes of the young.[7]

Glucose regulation is often examined in terms of absolute concentrations of glucose. But homeostasis is influenced by several hormones and substrates, and so a dynamic systems approach may be more appropriate.[8] For example, it is possible to describe the glucose regulatory system in a Cartesian way[9–11] (i.e., lines of best fit), but previous studies have shown that, individually, both glucose[12,13] and insulin[12] demonstrate nonlinear dynamic attributes. Published methods and models that examine glucose regulation[14,15] may use an understanding of homeostasis in their methodologies, but they do not quantify it.

Perturbed biological systems show large deviations from the norm, which revert to the basal state through metabolic processes acting over a period of time. In the case of glucose tolerance tests or a meal, the perturbation may last several hours. But even in the fasting state, the homeostatic principles still apply—there is no fixed point of basal "dwell" since insulin continues to be secreted in a time-variant way, and glucose responds to the insulin signal over an approximately 13 minute domain. A time series of fasting glucose and insulin data can be graphically represented as an x–y scatter plot, where the consistent time interval is indicated by a line between the relevant points. This then yields an attraction point or domain. As the system begins to depart from equilibrium, it is "attracted" back to this domain (sometimes referred to as a "strange attractor"[16]), which appears to have boundaries or limits. It is the time-dependent homeostatic space in which the basal feedback mechanism operates that we have analyzed.

To investigate the degree of alteration of homeostasis in those with diabetes, we have developed a modeled metric called Observed Variability And Lability (OVAL), a method whereby the variability of the glucose and insulin concentrations within the feedback domain can be quantified.

Methods

Subjects

Twelve patients (6 male) with type 2 diabetes mellitus [T2DM; median (range) age 35 (25–47) years, duration of diabetes of 7 (2–9) years, body mass index mean [standard deviation (SD)] 33 (3) kg/m^2, receiving conventional oral hypoglycemic treatment] and 27 controls [14 male, aged 38(30–53) years, mean (SD) body mass index 29 (5) kg/m^2] were studied. Subjects with diabetes did not take their medication on the day of testing.

Experimental Protocol

On the morning of the studies, patients attended the clinic, and fasting and blood sampling was undertaken in a resting supine position from a warmed distal forearm vein. Blood samples for the measurement of glucose and insulin concentrations were acquired every 2 min for 120 min.

Plasma was stored at -20 °C prior to batch analysis. All samples were analyzed in the same batch. Plasma insulin concentration was measured by radioimmunoassay using Novo human monocomponent insulin as standard and mono I-25 (Tyr A14) human insulin as tracer (Novo Biolabs, Cambridge, UK). Within-batch coefficient of variation by the method of duplicates was 12.5%. Blood glucose concentration was determined using the glucose-oxidase method with a coefficient of variation of 1.6% at 10 mmol/liter (YSI, Yellow Springs, OH).

The data come from studies that have been reported elsewhere[17] that were approved by the local ethics committee (Central Office for Research Ethics Committees), and written informed consent was obtained from the participants.

Analysis

We plotted the insulin–glucose time variant data as discrete observations, joined by lines representing 2 min intervals (**Figure 1**). This plot broadly defined the boundaries of the basal attraction domain or homeostatic area. From observation, we assumed that the total boundary of the attractor would be the ellipse that enclosed this area, and in order to avoid bias from outliers, we used the 95% confidence intervals (CIs) of the insulin and glucose to define, respectively, the height and width of the ellipse or the OVAL.

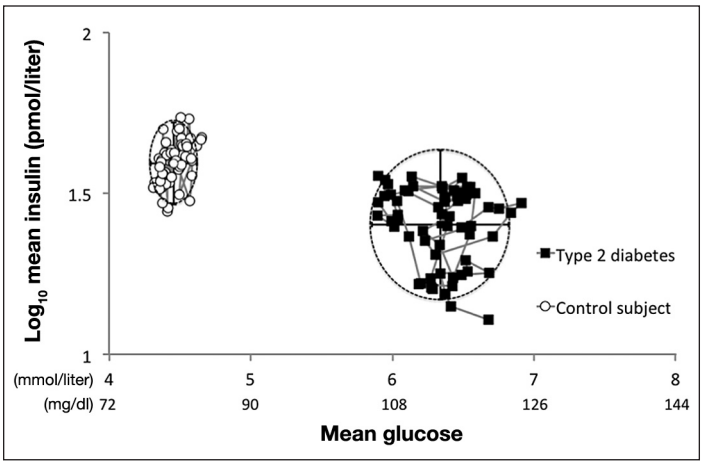

Figure 1. Glucose and $\log_{10}$ insulin in the basal state showing sequential 2 min sampling for 120 min for a T2DM patient (■) and a control (○) with consecutive observations joined by lines. Attraction point is the geometric mean insulin and mean glucose, and the limit cycle, 95% CI, is shown as bars. Outside the boundary, homeostatic mechanisms apply deterministically, and within the boundary, change is nonlinear, nonrepeatable, and nonpredictable.

To ensure a parametric distribution, the insulin data were $\log_{10}$ transformed. For the calculation of OVAL, an equal weighting for changes in insulin and glucose were required. This calculation ensured that, overall, the insulin domain was equally weighted with the glucose domain, thus avoiding the problem of units of measurement for glucose and insulin being different. When glucose is measured in mmol/liter and insulin in pmol/liter and $\log_{10}$ transformed, then the weighting ratio calculated was 1.1 (mg/dl formula: 18 unit change glucose mg/dl = 1 unit change $\log_{10}$ insulin pmol/liter × 1.1; mmol/liter formula: 1 unit change glucose mmol/liter = 1 unit change $\log_{10}$ insulin pmol/liter × 1.1).

The OVAL is calculated as 100 × ellipse area ([π × long axis × short axis]/4), where one axis is the 95% CI of basal glucose (mg/dl)/18 and the other axis is the 95% CI of basal $\log_{10}$ insulin (pmol/liter) × 1.1 (an OVAL calculator is available online at http://www.oval-calc.co.uk). It can be demonstrated using tangents of the axes that the 95% CIs do indeed form an ellipse—such that the calculated 45° CIs fall exactly on the boundary. A two-tailed Student's independent samples *t*-test was used to compare the two groups.

The primary analysis was performed on a 2 min sampling over 2 h periods (n = 61). To address the question of the minimum data set required to assess OVAL, we analyzed the ratio of the OVAL values between control and T2DM subjects for the data range n = 1 to 61.

Results

The variability of glucose and insulin was calculated separately using the mean 95% CI (SD) for the T2DM and control

groups. **Figure 2** shows the data for the mean $\log_{10}$ insulin, the mean glucose, and the OVALs for those with T2DM and controls. There was greater variability in mean glucose in T2DM subjects [T2DM 162 (77) mg/dl versus controls 45 (16) mg/dl; $p = .0003$]. The variability of insulin in T2DM subjects was increased but not significantly. The OVALs were significantly increased, by a factor of 4, in those with T2DM [T2DM 7.80 (3.83) versus controls 1.93 (1.00); $p = .0003$].

The control subjects cluster approximately between 45 and 90 mg/dl for glucose with tight glycemic variability. Insulin concentrations between the controls and the subjects with T2DM vary significantly by a factor of two orders of magnitude from the lowest minimum value to the highest maximum (0.14 to 2.11 in the $\log_{10}$ scale), exemplifying the requirement to transform the insulin data to avoid wide variations in insulin obscuring changes in the glucose axis.

An examination of the effect of the number of observations required to achieve a stable robust measure was undertaken in order to make an estimate of the minimum total time period required for sampling. We made the assumption that the observable difference between normal and diabetes subjects, expressed as a ratio, should be the marker of sufficient observations. These data are shown in **Figure 3**, where it can be seen that the estimate of the ratio (OVAL T2DM/OVAL control) for the full sampling period was approximately 4 and that this estimate degrades when less than approximately 26 samples are taken. This equates to 2 min sampling for 50 min.

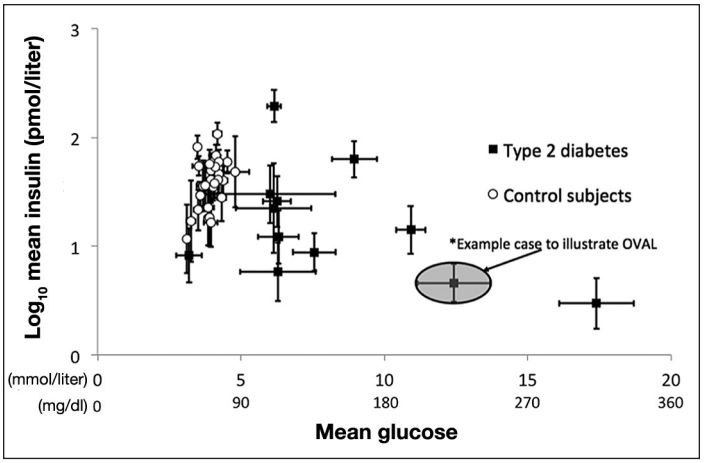

Figure 2. Twelve subjects with T2DM and 27 control subjects were studied. The center of the OVAL is defined as the geometric mean insulin and mean glucose with the "homeostatic boundary," defined as the 95% CIs. The sampling interval was 2 min for 120 minutes. The asterisk shows the example of the OVAL area shown only in one illustrative case for clarity.

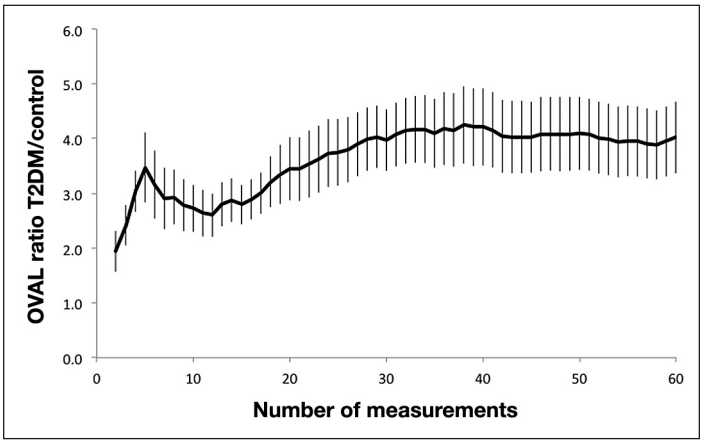

Figure 3. Ratio of the OVAL score in people with T2DM and controls for different numbers of sample measurements ($n = 2$ to 61). The black curve is the ratio, and the error bars are standard error of the mean.

Discussion

OVAL is a method to calculate the time-dependent homeostatic space in which the basal feedback mechanism operates. Its application to fasting data from subjects with T2DM demonstrated a significant difference of a four-factor change in the evaluation of homeostasis. It is known that the basal insulin and glucose values are not in steady state and that insulin in man is pulsatile in the fasting resting state.[8,18] Moreover, it is known that those with T2DM have more deranged insulin secretion, which may be irregular.[17] To date, there have been no quantifiable measures of homeostasis in diabetes, yet the gradual degradation of control mechanisms in T2DM is the hallmark of the condition. The OVAL method measures homeostasis, providing a quantified measure, which can be used in descriptions of degraded homeostasis in T2DM, impaired glucose regulation, and during therapeutic interventions.

Examination of the homeostatic system could lead to a better understanding of glucose dysregulation and elucidate the mechanisms that maintain euglycemia. For example, the increased area in the OVAL noted in T2DM is perhaps indicative of a lag in the insulin response to the prevalent glucose concentration as the feedback system becomes less dynamic. The plot of the insulin–glucose interdependence graph showed that variability is a natural state in controls and, although much increased in T2DM, is still present. Since the OVAL model is designed such that a one-unit change in insulin is equivalent to a one-unit change in glucose, we are confident that neither the changes in glucose

variability nor the changes in insulin variability have a dominant effect on the area of the OVAL. The dynamic interactions reflect a situation that can adjust rapidly to immediate perturbations—a heavily damped system would yield very small basal attractors but would result in a system that would not regain equilibrium for very many hours after a perturbation.[19,20]

The dynamic feedback process within homeostasis has also been examined by a number of groups, including the widely used HOMA model[21] that uses a simple understanding of the feedback characteristics to determine beta-cell function and insulin sensitivity. The latest interactive version of the HOMA model enables the dynamic feedback mechanism to be manipulated.[22] The HOMA model assumes a single point on the glucose–insulin domain in the basal state, while OVAL expands the concept into quantifying the normalized area of that domain. An issue with OVAL is that, in contrast to HOMA (which usually utilizes a single fasting sample for glucose and insulin estimation), OVAL requires frequent time series sampling of glucose and insulin concentrations during a basal period. We have illustrated OVAL using 2 min sampling over 2 h, but our analysis of the ratios obtained between normal and T2DM suggests that 50 min of 2 min sampling (26 observations) would yield substantially the same information. The reality is that there is a tradeoff between cost and accuracy. Two-minute sampling over a 2 h time frame may be accurate, but the optimal solution will have to be based on a pragmatic approach.

Conclusions

Deranged glucose–insulin homeostasis is the hallmark of diabetes. The OVAL model has the capacity to quantify this in the fasting state.

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