Glucose variability and diabetes complications: risk factor or marker? Can we disentangle the "Gordian Knot"?

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For several decades it has been well acknowledged that many metabolic diseases such as obesity, hypertension and diabetes are associated with and/or responsible for an increased risk of adverse cardiovascular events []. The harmful effects of such diseases or risk factors are proportional to their magnitude and duration and partly, but probably not fully, reverted during periods of weight loss and satisfactory control of blood pressure and/or glucose homeostasis, respectively []. However there is a large spectrum of increasing evidence that obese persons who experience body weight fluctuations, also referred to as "weight cycling" [], but also hypertensive people who have daily variations in blood pressure suffer from a greater risk of cardiovascular diseases than individuals with elevated but stable body weight and blood pressure []. For more than 10 years, similar observations have been made in persons with diabetes [] and, presently, there is an increasing number of reports that strongly suggest the role of glucose variability as aggravating risk factor for the development and progression of micro and macrovascular complications. Consequently, there arises the question as to whether two patients exhibiting similar average HbA1c levels over prolonged periods of time have identical risk for developing diabetes complications if one of the 2 patients exhibits relatively stable HbA1c and daily mean blood glucose concentrations whereas in the other one such metrics are broadly fluctuating around the same mean values irrespective of their high or small degrees of elevation. In addition, two other points deserve to be discussed. Firstly fluctuations in glucose homeostasis either on the long (HbA1c "cycling") or the short-term (within or between-day glucose variability) [] can reflect poor appropriateness of or compliance to medical care (nutritional and pharmacological measures). Secondly there is still an intensive debate between experts. Some of them consider that glycaemic fluctuations are potent causative factors of diabetes complications

while others believe that upward and downward glucose changes are simple markers of poor clinical outcomes. Whether one of the preceding explanation is preponderant or not compared to its counterpoint, the management of glucose disorders in diabetes should be aimed at reducing both the long and short-term glycaemic variability [] regardless of its mechanism. In addition, should the variability in glucose homeostasis be an accelerator for the harmful effects of sustained ambient hyperglycaemia [], it appears of interest to know beyond which degree of chronic hyperglycaemia it becomes mandatory to reduce the long and short-term glucose variability. As the risk of adverse events is not limited to cardiovascular complications but has to be extended to the risk of hypoglycaemic episodes, it is highly likely that the targets in terms of glycaemic variability should differ according to whether the main concerns are at an individual level driven by the development of long term complications or by the shorter perspective of frequent occurrence of acute adverse events such as severe repeated hypoglycaemia.

The objective of the present review is to disentangle the “Gordian Knots” that tie all the aforementioned issues. A clearer understanding of the role of temporal HbA1c and glucose cycling as causative factors or markers of chronic and acute adverse outcomes could help to gain better insight into the management of diabetes and prevention of its complications.

The facts

To clearly establish a causal relationship between the long or short-term glucose variability and the development or progression of adverse cardiovascular outcomes, it would be crucial to conduct controlled randomized trials (CRTs) mimicking those used for demonstrating the role of the overall glucose exposure, mainly assessed from HbA1c measurements, on diabetes complications. Such trials generally compared 2 groups of subjects randomly allocated to either an intensive or standard antidiabetic treatment over an initial “active” interventional period of several years, eventually-extended to a “post-interventional” follow-up of at least one decade such as in the UKPDS [], the VADT [] or the DCCT/EDIC []. Such studies led to the conclusion that intensified glucose-lowering therapies exerted beneficial effects that can be extended on the long-term, several years beyond the end of the period of intensive “active” management. Consecutively to these observations, the concept of metabolic memory was enounced and referred to as “glucose legacy”. Unfortunately and for several reasons that will be discussed later in this review, such beneficial effects were never assessed in prospective trials specifically designed for studying the impact of glucose fluctuations on “hard” outcomes such as fatal or non-fatal cardiovascular events. For the moment, all studies were only observational or based on the retrospective analysis of interventional trials not designed to investigate the aforementioned hypotheses. Consequently, our preliminary and introductive remark

will be to note that most studies cited below remain at a relatively moderate grade of clinical meaning despite their statistical significance [].

1) Relationships between long-term glucose variability and adverse clinical outcomes

This type of variability is an "umbrella" that covers a large spectrum of metrics that generally consist to assess visit-to-visit fluctuations of HbA1c at quarterly time intervals over prolonged periods of time. However the assessment of the long-term variability can be extended to spot blood glucose testing during a prespecified period of time on consecutive visits separated by weekly, monthly or quarterly time-intervals. Such protocols can be applied to fasting plasma glucose, postprandial glucose excursions or 7-point glycaemic profiles. The variability was further quantified by computing the temporal standard deviation of glucose (SD) or its %CV (coefficient of variation = 100 x SD/mean glucose). However it should be noted that SDs have magnitudes that are dependent on the magnitude of the data, thus explaining that the %CV is a better metrics of variability than the SD because it expresses the sample variability relative to mean of the sample. Despite this remark, some experts consider that the %CV is not independent of the mean because the mean appears as the unique component of the denominator of the fraction that defines the %CV. Therefore it has been proposed to use another formula, which is theoretically aimed at eradicating the influence of the mean. This parameter referenced to as the VIM (Variability Independent of the Mean) is given by the following equation: VIM = 100 x [SD/mean$^{\beta}$], where β is the regression coefficient between the natural logarithm of SD (Log SD) and the natural logarithm of the mean (Log mean) []. This coefficient is usually ranging from 0 to 1 according to whether there is no correlation (β = 0) or a positive correlation as an identity line (β = 1) when Log SD is plotted against Log mean. In these two extreme situations that are rarely encountered the value of the VIM is equal to either the %CV or the (SD x 100) when β values are of 1 or 0, respectively. Despite these remarks, it appears in fact that in most studies the VIM does not provide any additional information in terms of statistical significance when compared with the %CV [] that has the main advantage to be easily understandable by healthcare practitioners [].

The pioneering studies

In the early years of the current century, Kilpatrick et al described for the first time an association of the HbA1c variability with the development of diabetic retinopathy by analyzing the datasets of the DCCT []. However the HbA1c variability was closely related with the mean HbA1c of all patients (r = 0.55), thus leaving us with the mixed feeling that the aforementioned observation could simply

reflect the well-known association between the total glucose exposure and the risk for developing microvascular complications. In addition, the same investigators [] failed to find any association when the risk of diabetic retinopathy was tested against quarterly 7-point glycaemic profiles recorded over 3 consecutive days. Such controversial results did not help to clarify the relationship between diabetic microvascular complications and the long-term glycaemic variability.

The 2015 meta-analysis

Several years later, Gorst et al published a systematic review and meta-analysis of 7 and 13 studies conducted in type 1 and type 2 diabetes, respectively []. The authors found that higher HbA1c variability was associated with higher risk of cardiovascular events: risk ratio = 1.98 [CI95%=1.39 – 2.82] and 1.27 [1.15 – 1.40] in type 1 and type 2 diabetes, respectively. However, most studies included in this meta-analysis suffered from a lack of adjustment for potential confounders and poor definitions of HbA1c variability, thus rendering the results questionable.

The most recent studies

Taking into account the limitations of the previous aforementioned studies, it seems of importance to report the results of several studies published in the recent years with the aim to deciphering whether there exists or not an association between the long-term glycaemic variability and cardiovascular diseases in diabetes. Even though these latest studies were similar to the post hoc analysis of earlier clinical trials, they have the advantage to have been conducted in highly selected populations with rigorous inclusion and exclusion criteria, in the context of prospective trials in large size populations over long periods of follow-up. However as these studies seem to differ in their degree of relevance, the following stratification is used:

a) The less relevant studies

- The post hoc analysis of the ALLHAT study [] that was conducted over a median follow-up of 5 years in a large cohort of 4,892 individuals with and without diabetes has tested the risk for developing cardiovascular events against the visit-to-visit variability of fasting blood glucose (FBG) after this parameter has been divided into quartiles with the lowest serving as reference. Glycaemic variability was only determined from 3 FBG measures (a number that appears rather small) at 24-month intervals and further computed as 4 metrics: the popular SD and %CV and the less classical VIM and ASG (Average Successive Variability). In the population considered as a whole, the participants in the highest quartile showed a significant increase in all-cause mortality when compared with those in the lowest quartile. Surprisingly, these results were only significant in the people without diabetes for all-cause

deaths, but not cardiovascular events. From a general point of view, one can wonder how the glycaemic variability can be only computed from 3 FBG measurements and why the potential interplay between mean FBG and its variability was never evoked and tested []. Consequently the results of this study remain questionable.

- By analyzing the datasets of 54,803 subjects (≥ 70 years of age) with diabetes who were included in the Health Improvement Network (THIN), Forbes et al [] found that the overall mortality rate has a J-shaped distribution when plotted against mean HbA1c levels and a positive increasing relationship with glycaemic variability of HbA1c. However these results remain poorly informative for several reasons. Firstly, types of diabetes were not accurately defined because the coding of diabetes was surprisingly not reliably registered in the database. Secondly, the HbA1c data were not collected in a controlled time-specified way. Thirdly, the causes of deaths were never indicated and therefore, it is impossible to know whether the enhanced mortality risk with the increasing long-term glycaemic variability score is due mainly to fatal cardiovascular events or other diseases. Consequently, it is difficult to agree with the conclusion of the authors who suggest that the long-term glycaemic variability, as assessed from variability in HbA1c over time, might be an important risk factor in understanding mortality risks in older people with diabetes.

b) The studies with less limitations and improved relevance

Three of them were published during the last two years and seem to indicate that reducing mean HbA1c and attenuating its variability at the same time is better than limiting therapeutic interventions to average HbA1c alone. The first study was based on the analysis of the datasets of the English Clinical Practice Research Datalink (CPRD) conducted in a large population of 58,832 patients with type 2 diabetes, who were tested for HbA1c variability during the period between 2006-2009 and further followed for clinical outcomes from 2010 to 2015. By using these data, Critchley et al [] demonstrated that after selecting two groups of patients according to whether the average HbA1c was low (≤ 6.58) or high (> 7.91%) the risk of all-cause mortality increased progressively in both groups with worsening HbA1c variability. When the patients were separated into 2 groups by low or high HbA1c variability (coefficient of variation ≤ 4.71% or high > 11.40%, respectively) the risk of all-cause mortality with worsening HbA1c became only significantly elevated when the mean HbA1c was >8.88%. Such results suggest that reducing the HbA1c absolute level is of importance, but means also that focusing the targets on both stability and absolute levels of HbA1c adds strength to the therapeutic strategy. However the results observed by Critchley et al remain questionable because there was a time lag of several years between the assessment

of outcomes at end-point and the measurements of HbA1c metrics (mean and variability) during the run – in period of the study.

Consequently, one of the post hoc analyses of the data provided by the ACCORD Trial [] is of major interest when the 9,153 participants (4,728 and 4,755 in the standard and intensive therapy groups, respectively) were tested for HbA1c CV, VIM and ARV (Average Real Variability). After dividing the intensive and standard therapy groups into tertiles of mean HbA1c and stratifying the subgroups as obtained by tertiles of glycaemic variability (VIM) the cross tabulation analysis of mean HBA1c and long-term glycaemic variability (VIM of HBA1c) showed that the incidence of all-cause mortality increased with worsening mean HbA1c and with increasing VIM of HbA1c. Therefore the highest incidences of all-cause mortality were observed in the group combining the highest visit-to-visit variability in HbA1c and the highest mean HbA1c (figure 1). In addition another secondary analysis of the ACCORD Trial [A] showed a significant graded association between the long-term variability in mean HbA1c and the risk of heart failure such that participants in the highest quintile of HbA1c variability had approximately a two fold risk of heart failure when compared with those in the lowest quintile (event rate = 6.2 vs 3.2%, respectively). Similar associations between visit-to-visit HbA1c variability- and the risk of lower-extremity amputation were also reported in 30,031 patients with type 2 diabetes. Multivariate adjusted Hazard Ratios for such adverse events increased from 1 to 1.88 across quartiles of HbA1c, CV (P trend = 0.012) [B].

Bringing all these data together, it seems that the long-term glycaemic variability is strongly associated with the risk of adverse clinical outcomes. However, the debate is yet widely open because no one can state that this glycaemic disorder is rather a causative factor for either cardiovascular events or all-cause mortality than a simple marker of unsatisfactory adherence to lifestyle measures and/or pharmacologic treatments [].

2) Relationship between short-term glycaemic variability and cardiovascular outcomes

The short-term glycaemic variability is defined as rapid within or between-day upward and downward fluctuations of glucose concentrations []. In the seventies several parameters such as the Mean Amplitude of Glycaemic Excursions (MAGE) [C] and the Mean Of Daily Differences (MODD)[] were proposed to estimate the within and between-day glycaemic variability, respectively. At that time these parameters were mainly calculated from the self-monitoring of blood glucose because the technology of continuous glucose monitoring was only available using a continuous intravenous blood sampling over limited periods of time, usually a few hours, after admission at ward units in hospital settings specialized in the investigation of glucose homeostasis [E]. When in the early years of the present century the continuous glucose monitoring (CGM) became available on an ambulatory basis over periods of several days and progressively over two weeks [] with the limited inconvenience of painless insertion of subcutaneous glucose sensors, new metrics were developed [].

The profusion of metrics rapidly created a certain degree of confusion [], the most important remark being that many experts attempted to develop a large spectrum of more and more sophisticated indices with the disadvantage that they evolved to be less and less understable by patients and healthcare professionals. Therefore simple metrics should be recommended for the assessment of short-term glycaemic variability []. From a practical point of view, we should be grateful to the International Conference on the Use of Continuous Glucose Monitoring [F] for having adopted the coefficient of variation for glucose as the most appropriate index for assessing the within-day glycaemic variability, with a cut-off threshold value of 36% to separate stable from labile glycaemic control [G]. At present, there is no study providing full evidence that the short-term glycaemic variability is a causative risk factor for cardiovascular complications in diabetes []. However, absence of evidence is not synonymous of evidence of absence because a number of studies suggest that the short-term glycaemic variability could play a role in the development of adverse clinical outcomes.

a) Lessons from observational studies

As the oxidative stress and proinflammatory or prothrombotic cytokines are key players in diabetes complications [] our seminal observation published in 2006 represents a good start by demonstrating that the activation of oxidative stress assessed from the urinary excretion rate of the free 8-isoprostaglandin F2α (8-isoPGF2α) is strongly and positively correlated with the instability assessed from the MAGE ($r= 0.86$, $P<0.001$) in a limited group of 21 poorly controlled type 2 diabetic patients (mean HbA1c ± SD = 9.67 ± 1.30%) treated with oral antidiabetic agents alone []. Ceriello et al further confirmed such results when they found that oscillating glucose concentrations are more deleterious to vascular endothelial function than sustained hyperglycemia by using the glycaemic clamp methodology in healthy subjects and non-insulin treated patients with type 2 diabetes []. However the clinical relevance of such observations was apparently attenuated when Wenthold et al failed to find any relationship between the urinary excretion rate of 8-isoPGF2α and MAGE in relatively well controlled patients with type 1 diabetes (mean HbA1c ± SD = 8.10 ± 0.90%) []. These divergent results raise several questions, but two main explanatory factors can be proposed. Firstly, the discrepancies can be due to the differences observed in HbA1c levels. Should this explanation be true, it can be suggested that patients not sufficiently controlled in terms of chronic glucose exposure are more prone to suffer from harmful metabolic effects of excessive short-term glucose variability than those who are not exposed to abnormally high ambient hyperglycaemia. However this hypothesis remains unproven and questionable, thus leading to a more likely proposal based on the intrinsic inhibitory effect of exogenous insulin on inflammation, thrombosis and oxidative stress []. For instance, in insulin-requiring type 2 diabetes or in type 1 diabetes, the urinary excretion rate of 8 isoPGF2α was found within the normal range observed in healthy non-diabetic controls []. This type of observation

suggests an inhibitory effect of insulin on the activation of the oxidative stress and thus explaining the lack of correlation between the 8 isoPGF2α and the MAGE, which was observed in type 1 diabetes []. In support to this hypothesis, it should be noted that a drastic reduction in the urinary excretion rate or 8- isoPGF2α was observed when persons with type 2 diabetes treated with oral hypoglycaemic agents are shifted to insulin treatments [].

b) Lessons from interventional studies

A few trials were conducted during the last past years but none of them led to convincing results. One example is given by the FLAT-SUGAR trial [], which was designed to test whether an add-on therapy with a glucagon like peptide 1 receptor agonist-based regimen delivered as twice daily injections of exenatide associated to an ongoing basal insulin treatment in persons with an insulin – requiring type 2 diabetes can reduce the short-term glycaemic variability and subsequently can improve markers of cardiometabolic risk. In this study [] the exenatide arm was compared with a group of participants treated with a basal-bolus insulin regimen. Small decrements in glycemic variability from baseline to end point were observed but remained limited to the %CV and MAGE in the exenatide group. No improvements occurred with the basal-bolus insulin regimen after 26 weeks of follow-up. Unfortunately the small reduction in short-term glycaemic variability in the exenatide group was not associated with any improvement in plasma levels of IL-6, CRP and changes in urinary excretion rates of 8-isoPGF2α. Measurements of all these metrics were included in the protocol with intend to have a sneak peek on the potential effects of GV reduction on the risk factors or markers of cardiovascular diseases. As both arms in the FLAT-SUGAR Trial were treated with insulin, the negative findings concerning these metrics are not surprising [] and they even give an additional support for the existence of an inhibitory action of insulin on inflammation, thrombosis and activation of oxidative stress []. Similar comments can be made after the investigations of the HEART 2D study [], which failed to find any difference in terms of cardiovascular outcomes when patients with type 2 diabetes were assigned to either a conventional basal insulin strategy or an insulin regimen aimed at reducing postprandial glucose excursions, i.e. the short-term glycaemic variability [] with three daily injections of rapid acting insulin analogues before meals. Consequently, it appears that the future of randomized controlled intervention trials aimed at testing the effect of short-term glycaemic variability should avoid the use of insulin preparations in either or both groups in order to escape to any misinterpretation in data analysis at end-point of the study [].

c) Lessons from studies in critically ill patients

Hyperglycaemia has been reported to be associated with increased mortality in critically ill patients [a] while targeting euglycaemia [b] and glycaemic variability [c] seems to improve the prognosis of

such patients. In a large population of individuals with (n = 942) and without diabetes (n = 3142) who were admitted in intensive care units for severe clinical conditions, Krinsley demonstrated that the percentage mortality was steadily increasing with worsening mean glucose levels during their stay in the hospital [d]. However, and surprisingly, the relationship was only observed in patients who were free of any glycaemic disorder before their admission at the hospital. When these people were stratified by quartiles of glucose concentrations, there was a significant positive association between increments in glycaemic variability and mortality [d]. Once again, and surprisingly, the association was not observed in those with diabetes [d]. As a consequence, such observational studies do not help to define the optimal blood glucose targets that should be achieved in general populations who are hospitalized in intensive care units and even in such selected populations at high risk such as those suffering from diabetes [e]. However the use of CGM in intensive care units seems to be recommended because this technology can contribute to achieve a safer glycaemic profile [f], which in turn can permit to minimize the risk of hypoglycaemia by reducing the glycaemic variability in patients who must benefit from an averaged tight glucose control.

3) Relationship between the short-term variability and hypoglycaemia

The ACCORD study has established that trying to achieve a too stringent glycaemic control in terms of mean HbA1c resulted in increased frequency of hypoglycaemia in type 2 diabetes []. Several years before, the DCCT Research group in type 1 diabetes reported that the risk of severe hypoglycaemia was increasing continuously with lower HbA1c values []. A few years later, with the emergence of the CGM technology as a powerful tool for investigating the short-term glycaemic variability, it rapidly appeared that excessive intra-day glucose fluctuations were associated with higher risk of hypoglycaemia in patients with diabetes and more specifically in those exhibiting a near normal glucose control in terms of average HbA1c levels []. More recently, we used the CART methodology to decipher whether the short-term glycaemic variability is more significant than the mean daily glucose concentration to predict the risk of unequivocal hypoglycaemia (glucose value < 3 mmol/L, i.e. 54 mg/dL). This analysis showed that the short-term glycaemic variability assessed from the %CV and the mean daily glucose concentrations were not equivalent contributors to hypoglycaemia []. For instance, we found that the positive relationship between the time spent below 3 mmol/L and the %CV was more statistically significant than the inverse relationship observed when the time spent below 3 mmol/L was plotted against the mean daily glucose concentration (figures 2a and 2b). A few months later Ahmadi et al have reported similar relationships. In addition, when the daily glucose value was < 7.8 mmol/L, it is required to achieve a %CV below 34% for minimizing the risk of hypoglycaemia, this threshold value being very close to the cut-off value of 36% that separates stable

from labile diabetes [G]. Such results indicate that patients with diabetes should try to reach a subtle equilibrium between the chronic ambient hyperglycaemia and its fluctuations around a mean glucose value in order to reduce both the chronic risk for developing diabetic complications in the long-term and the acute risk of hypoglycaemia in the short-term, respectively. However recent studies demonstrated that parallel reductions in HbA1c, short-term glycaemic variability and frequency of hypoglycaemia could be simply obtained in CGM users with diabetes regardless of the modality of insulin delivery method [H à M]. As an example, the investigators of the DIAMOND Study [] found that among patients with type 1 diabetes treated with multiple daily injections of insulin, CGM users exhibited a significant improvement in HbA1c levels from 8.6 to 7% at end-point, in %CV from 42% to 38% ($p < 0.001$) and in the risk of hypoglycaemia irrespective of the selected alert threshold: 70 ($p < 0.002$), 60 ($p = 0.02$) or 50 mg/dL ($p = 0.001$). Considering these studies as a whole, there is increasing arguments for enouncing that the two objectives of limiting the risk of hypoglyacemic episodes and minimizing the risk of long-term complications are not antinomic but complementary provided that the short-term glycaemic variability is reduced concomitantly with the diminution of the mean glucose exposure. In addition, this type of remark raises the question as to whether frequent hypoglycaemic episodes participate to the occurrence of adverse clinical outcomes. At present, despite an abundant literature that has reported that hypoglycaemia, especially when they are severe, are associated with the risk of both acute cardiovascular events or chronic diabetes complications [N à U), their potential deleterious involvement to promote cardiovascular events remains limited to surrogate outcomes [V] such as the induction of proarythmic cardiac disorders [], endothelial dysfunction [] and enhanced production of prothrombotic, proinflammatory or platelet proaggregant mediators or markers []. Consequently, it has never been clearly established that hypoglycaemia directly contributes to “hard” adverse outcomes [] and thus it is possible that hypoglycaemic episodes just could be markers of vulnerability to such events [N,g]. Therefore should we consider that hypoglycaemic epidodes be intermediary links in the catenary chain from short-term variability to adverse clinical outcomes, it seems that there is supportive evidence for the first step (from short-term glucose variability to hypoglycaemia) and a lower evidence for the second step (from hypoglycaemia to adverse outcomes).

4) Relationship between the short-term glycaemic variability and postprandial glucose excursions.

As mentioned above, hypoglycaemia or downward glucose fluctuations are strongly associated with the short-term glycaemic variability. However the latter glycaemic disorder is also the result from upward glucose fluctuations, which are in turn the consequence of postprandial excursions.

Therefore one of the pending questions is to quantify the contribution of postprandial glucose increments to the overall glycaemic variability. To address this issue we have used the data collected in the 30 persons with type 2 diabetes, who were included in the OPTIMA trial []. This study was initially designed to compare the effects of sitagliptin and vildagliptin, two DPP-4 inhibitors given as add-on therapy to an ongoing treatment with metformin. After 8 weeks of follow-up, several markers of glycaemic control were measured. The results (expressed as changes from baseline) showed that the δMAGE and δAUCpp (i.e. the postprandial increments area from premeal glucose values) were strongly and positively correlated ($R^2 = 0.48$, $p < 0.0001$, $n = 30$) (figure 3). As the results were expressed as changes (δ) with δAUCPP and δMAGE as explanatory (X) and dependent variables (Y), respectively, it can be concluded that in this regression situation 48%, i.e. approximately one half of the overall short-term glycaemic variability is attributable to the amplitude of postprandial glucose excursions. Consequently, there are some reasons to think that upward and downward fluctuations around the mean daily glucose value contribute equally to the short-term glycaemic variability. A large number of studies have shown that treatments aimed at smoothing postprandial glucose excursions such as short-acting GLP-1 RAs [] or insulin analogs [] concomitantly reduce the overall glycaemic variability. By contrast, the short-term glucose variability remains usually unchanged when pharmacologic strategies such as the implementation of basal insulin regimens alone in type 2 diabetes are only exerting a downward translation of the 24-h glycaemic profiles without producing any reduction in the magnitude of postprandial glycaemic excursions []. Therefore reducing postprandial excursions appears as one of the main recommendations for exerting a beneficial impact on the overall glycaemic variability. In addition the activation of oxidative stress, which has been described by Ceriello during postprandial periods [] appears as one of the two contributors to the more general enhanced production of oxidative stress mediators , which is associated with glycaemic disorders [].

Attempting to untangle the “Gordian knot”

As developed across this review, the past few years were marked by cumulative numbers of publications that have suggested or demonstrated the existence of close interrelationships between glucose variability and diabetes complications. However, these two variables were more described as to be associated rather than as to be linked by a cause-effect relationship. This is mainly due to the fact that most data were provided from retrospective analyses of clinical studies that were not specifically designed for deciphering whether long or short-term variability plays a decisive role in the development or progression of diabetes complications. Consequently, we are still awaiting specific randomized interventional controlled trials where the effect of a low- glycaemic variability group

would be tested against a high-glycaemic variability group, everything else being equal. Therefore such a study should be conducted with a similar control of the ambient hyperglycaemia in both groups, over a long period of several years with primary and secondary end-points selected among "hard" outcomes such as cardiovascular events and all-cause mortality. In addition, the designers of such trials should not forget that some antidiabetic drugs such as the GLP-1 RAs or SGLT2 inhibitors can have a specific potential effect on the onset of cardiovascular diseases [] and that others treatments such as those with insulin preparations can exert antithrombotic, anti-inflammatory or antioxidant effects []. Therefore, the use of these agents in such trials should be either avoided or randomly distributed in equivalent proportions in the 2 groups. Considering all these difficulties, it seems that it will be almost impossible to conduct such a trial in a large number of participants who in addition should wear a CGM over the entire period of follow-up. Consequently, the Gordian knot is far from being untangled and at present we are only allowed to formulate a few recommendations to classify the effects of glycaemic variability according to a decreasing graded scale of evidence from high supportive to no evidence, with one intermediary stage referred to as medium/low supportive evidence. In order to clarify the debate as far as possible, we have individualized two categories of effects for the long-term, short-term variability and for its composite risk factor, the hypoglycaemia: (i) the effects only limited to cardiometabolic markers and (ii) those extended to "hard" clinical outcomes. As preliminary remark, the mention "medium/low supportive evidence" should be usually attributed to the first category because the results were only obtained from observational studies conducted on the basis of pathophysiological concepts/hypotheses. For the second category, the word "associations" should be substituted for effects because prospective interventional trials are lacking. However, several studies included in this category can be labeled to as showing high supportive evidence because based on the retrospective analysis of data collected during the long-term follow-up of either well-conducted trials or unequivocal observational studies. Taking into account all these remarks, the interrelationship can be summarized as follows (figure 4a and 4b), even though this proposal remains subject to discussion.

a) Evidence for cause-effects on cardiometabolic markers (figure 4a) with a medium/low grade of evidence. Both the short-term glycaemic variability and hypoglycaemia seems to activate the productions of cardiometabolic mediators or markers. By contrast, there is no study indicating that the long-term glycaemic variability has a stimulatory effect on such productions, but absence of evidence is not synonymous of evidence of absence.

b) Associations assessed on the basis of "hard" outcomes (figure 4b). There is high supportive evidence that long-term glycaemic variability and hypoglycaemia are associated with "hard" clinical outcomes, but no answer can be given to the question as to whether these two glycaemic disorders

are risk factors or simple markers of adverse outcomes. For the short-term glycaemic variability, the absence of evidence is simply due to the lack of interventional study aimed at testing its impact on "hard" clinical outcomes.

c) Interrelationships between the three glycaemic disorders.

For the moment there is medium/low supportive evidence that hypoglycaemia are associated with both the short and long-term variability, but the hypothesis that hypoglycaemia is an intermediary link in a catenary model from short and long-term glucose variability to "hard" outcomes remains speculative. In addition no information is available concerning the existence of likely interplays between short and long- term variability.

At the end of this review it is noteworthy that the role of the different components of glycaemic variability in the development of diabetic complications has been more accurately investigated during the last past few years than during the preceding decades, even though many points still remain unclear. An example of this duality between accuracy and cloudiness can be given by the discrepancies between the targets recommended for the short-term variability or hypoglycaemia and the long-term variability (figure 5). For the two former clear targets were defined: %CV < 36% for the short-term variability [], threshold of alert of 70 mg/dL for defining any hypoglycaemic episode, with a percentage of time below 70 mg/dL < 4% and a cut-off glucose value of 54 mg/dL for clinically significant hypoglycaemia with a percentage time below 54 mg/dL < 1% []. Unfortunately such recommendations are missing for the long-term glycaemic variability. This failure is probably due to the fact that the basis and the time interval for its computation were never precisely defined between visit-to-visit changes in HbA1c, spot plasma glucose measurements or determinations of multiple point glycaemic profiles [].

Concerning the questions raised in the introduction of this review, in all persons with diabetes it should be recommended to maintain the short-term glycaemic variability within reasonable limits, ideally below a %CV of 34-36 % [] whatever the level of overall glucose exposure. This recommendation is mandatory when it comes to persons experiencing frequent/severe hypoglycaemia or to those in whom a tight glycaemic control has to be achieved in terms of HbA1c. In addition we are presently unable to give a response to the question as to whether HbA1c "cycling"is a non-acquired disorder or the consequence of an intermittent poor compliance to therapeutic measures [].

Legends of figures

Figure 1: Incidence of all-cause mortality as a function of increasing visit-to-visit HbA1c variability (tertiles of VIM, variability independent of Mean) in the lower, medium and upper tertiles of mean HbA1c. Data computed from persons with type 2 diabetes who were allocated to the intensively-treated group of the ACCORD trial (from reference……)

Figure 2: Relationship between the time spent below 54 mg/dL versus the mean daily glucose concentration (figure 2a) and the coefficient of variation for glucose (figure 2b) (from reference….)

Figure 3: Relationship between changes in postprandial glucose increments (δAUCpp) and changes in short-term glycaemic variability (δMAGE) from baseline to end-point after a 8-week period of treatment with gliptins in 30 patients with type 2 diabetes included in the OPTIMA study (from reference….)

Figure 4: Interrelationships between metrics of glycaemic variability (short, long-term variability and hypoglycaemia) and cardiometabolic markers/mediators (figure 4a) or "hard" clinical outcomes (figure 4b).
In figures 4a et 4b the interrelationships are defined as causal effects and associations, respectively. Solid thick and thin arrows indicate high supportive and medium/low interrelationships (effects of associations), respectively. Dotted arrows indicate the absence of interrelationship or lack of studies.

Figure 5: Targets for metrics of glycaemic variability (short, long-term variability and hypoglycaemia). Targets for hypoglycaemia should be completed by recommendations concerning the percentages of time spent below range (TBR): < 4% and < 1% when the thresholds are set at 70 and 54 mg/dL, respectively.
Recommendations have never been formulated for the long-term glycaemic variability because the consensus is missing on its assessment: quatrly visit-to-visit changes in HbA1c, spot fasting plasma glucose at regular time intervals, monthly or quarterly multiple-point glycaemic profiles.

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Figure 1

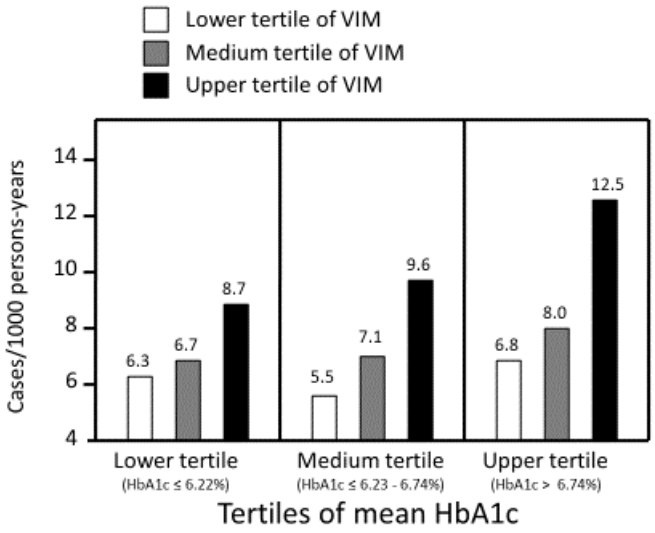


Figure 2a and 2b

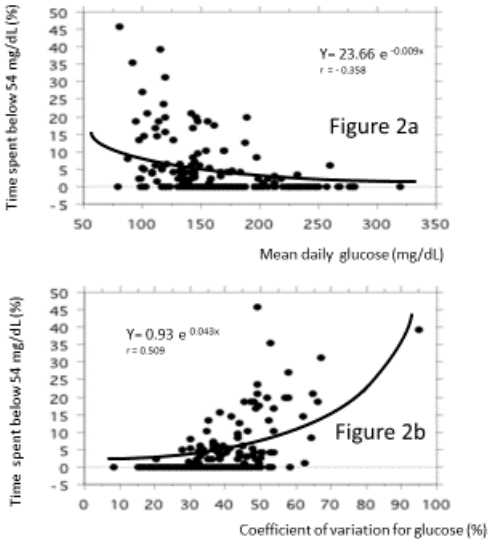


Figure 3

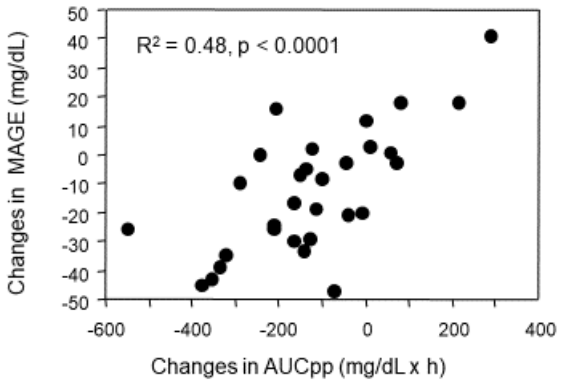


Figure 4a and 4b

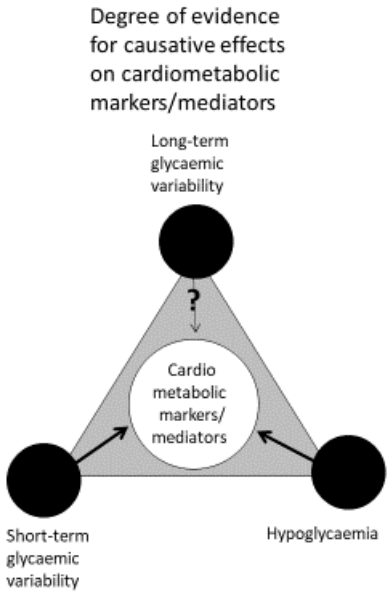


Figure 4a

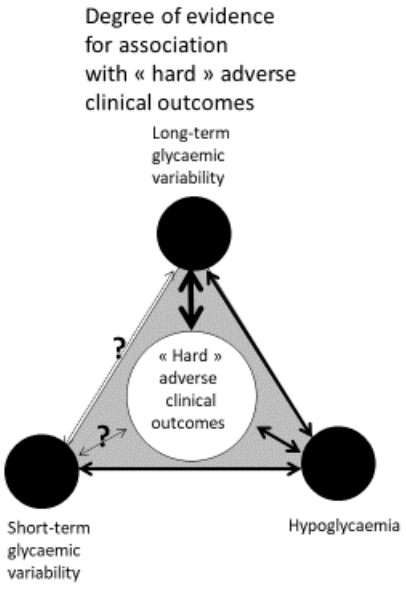
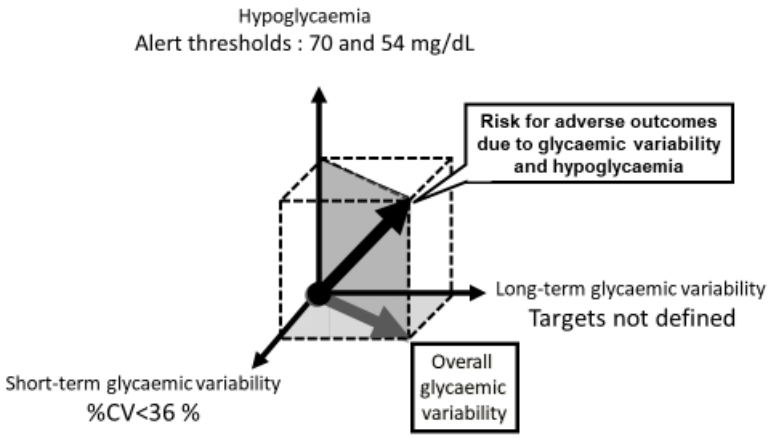


Figure 4b

Figure 5


Hypoglycaemia
Alert thresholds : 70 and 54 mg/dL
Risk for adverse outcomes due to glycaemic variability and hypoglycaemia
Long-term glycaemic variability
Targets not defined
Overall glycaemic variability
Short-term glycaemic variability
%CV<36 %