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## **Glucose variability and diabetes complications: risk factor or biomarker?**

### **Can we disentangle the “Gordian Knot”?**

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## **Abstract**

« Variability in glucose homoeostasis » is a better description than « glycaemic variability » as it encompasses two categories of dysglycaemic disorders: i) the short-term daily glucose fluctuations and ii) long-term weekly, monthly or quarterly changes in either HbA1c, fasting or postprandial plasma glucose. Presently, the relationship between the “variability in glucose homoeostasis” and diabetes complications has never been fully clarified because studies are either observational or limited to retrospective analysis of trials not primarily designed to address this issue. Despite the absence of definitive evidence from randomized controlled trials (RCTs), it is most likely that acute and long-term glucose homoeostasis “cycling”, akin to weight and blood pressure “cycling” in obese and hypertensive individuals, are additional risk factors for diabetes complications in the presence of sustained ambient hyperglycaemia. As hypoglycaemic events are strongly associated with short- and long-term glucose variability, two relevant messages can be formulated. Firstly, due consideration should be given to avoid within-day glucose fluctuations in excess of 36% (coefficient of variation) at least for minimizing the inconvenience and dangers associated with hypoglycaemia. Secondly, it seems appropriate to consider that variability in glucose homoeostasis is not only associated with cardiovascular events but is also a causative risk factor via hypoglycaemic episodes as intermediary step. Untangling the “ Gordian Knot”, to provide confirmation about the impact of variability in glucose homoeostasis and diabetes complications remains a daunting prospect.

**Key words:** Diabetes complications; Glucose variability

## Introduction

It has been well acknowledged over several decades that diseases and/or risk factors such as obesity, hypertension, dyslipidaemias and diabetes are associated with and/or contribute to an increased risk of adverse cardiovascular complications [1-5]. The deleterious impact of such clinical conditions is proportional to their magnitude and duration and partly reversible during periods of weight loss [6-8], control of blood pressure [9,10], dyslipidaemia [11,12] and glucose homoeostasis, respectively [13-16]. There is increasing evidence that persons who experience fluctuations in body weight (“weight cycling”) [17-22] or blood pressure [23-27] suffer from a greater risk of cardiovascular diseases than individuals with elevated but stable body weight and blood pressure. Similar observations have been made in persons with an increasing number suggesting that glucose variability is a risk factor or an accelerator for the development and progression of micro- and macrovascular complications [28-36]. Consequently, the question is whether two individuals with a similar HbA1c levels over prolonged periods will have identical risk for developing diabetes complications if one of the persons exhibits increased glucose variability. In addition, two other points deserve to be discussed. Fluctuations in glucose homoeostasis either long- term (HbA1c “cycling”) or short-term (within or between-day glucose variability) [37] can reflect poor appropriateness of or compliance to medical care (nutritional and/or pharmacological). Secondly, there is intensive debate between experts who consider that glycaemic variability is an independent risk factor of diabetes complications [38,39] and those who merely believe that it is a reflection of poor glycaemic control [40,41] or who even refute any association with diabetic complications [42]. Current management priority is to reduce both the long and short-term glycaemic variability whilst avoiding hypoglycaemia [36]. It is being suggested that short-term glucose variability may be an accelerator for the harmful effects of sustained ambient hyperglycaemia [28]. Therefore it is important to know beyond which degree of dysglycaemia it becomes mandatory to reduce either the long and/or short-term glucose variability [31,43-47]. As the risk of adverse events is not limited to cardiovascular complications but has to be extended to hypoglycaemic episodes [48-52], the glycaemic variability targets may differ according to whether the main concerns are the development of long term complications and/or repeated acute hypoglycaemic events .The objective of the present review is to attempt to disentangle the “Gordian Knots” that link all the aforementioned issues. A clearer understanding of the relative roles of the different components of dysglycaemia either chronic stable hyperglycaemia or long- and short-term “glucose cycling” as causative factors of adverse outcomes could help to better manage diabetes and prevent its complications.

## What is observed?

In order to clearly establish a causal relationship between long or short-term glucose variability and the development or progression of adverse cardiovascular outcomes, it would be essential to conduct controlled randomized trials (CRTs). These studies would need to be long-term where the overall glycaemic control is comparable between the groups (average HbA1c, mean glucose levels), which otherwise would differ by either long-term (e.g. coefficient of variation for HbA1c, %CV HbA1c, calculated from quarterly determinations of HbA1c) or short-term (e.g. %CV for glucose, Mean Amplitude of Glycaemic Excursions, Mean Of Daily Differences) glucose variability. The UKPDS (United Kingdom Prospective Diabetes Study) [14], VADT (Veterans Affairs Diabetes Trial) [53] and the DCCT/EDIC (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) [15] studies were long-term comparing intensive versus conventional treatment and not specifically designed for studying the impact of glucose fluctuations on “hard” outcomes such as fatal or non-fatal cardiovascular events. For the present time, all cardiovascular outcome trials (CVOT) were observational or based on retrospective analysis of interventional trials [16]. Consequently, our preliminary and introductory remarks will indicate that most studies cited below are at a relatively moderate grade of clinical meaningfulness to address the hypothesis that glucose variability is an independent risk factor for cardiovascular disease in the diabetic population despite their statistical significance [54-56].

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

This description “long-term” glucose variability is an “umbrella” term, which usually refers to the assessment of visit-to-visit fluctuations of HbA1c at quarterly time intervals over prolonged periods of time. However, the assessment of the long-term variability can also be extended to spot blood glucose testing such fasting plasma glucose, postprandial glucose excursions or 7-point glycaemic profiles conducted on visits at weekly, monthly or quarterly intervals [36,37,57,58]. The variability has been further quantified by computing the temporal standard deviation of glucose (SD) or its derivative %CV (coefficient of variation =  $100 \times \text{SD} / \text{mean glucose}$ ). As the SD is dependent on the magnitude of the data, the %CV is regarded a better metrics of variability as it expresses the sample variability relative to mean of the sample [59]. However 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 an other formula, first used for estimating the visit-to- visit variability in blood pressure [60-62], has recently been proposed with the aim to theoretically eradicate the influence of the mean glucose on the %CV for glucose. This parameter is the Variability Independent of the Mean ( $\text{VIM} = 100 \times [\text{SD} / \text{mean}^\beta]$ , where  $\beta$  is the regression coefficient between the natural logarithm of SD

(Log SD) and the natural logarithm of the mean (Log mean). This coefficient usually ranges from 0 to 1 according to whether there is no correlation ( $\beta = 0$ ) or a positive correlation as an identity line ( $\beta = 1$ ) 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  $\beta$  values are of 1 or 0, respectively. Despite these remarks, in most studies the VIM does not provide any additional statistical information when compared with the %CV [61,63,64] that has the main advantage to be easily understandable by healthcare practitioners [37].

### **The pioneering studies**

During the early years of this century, Kilpatrick et al first described an association between HbA1c variability and the development of diabetic retinopathy after having analyzed datasets of the DCCT [39]. The HbA1c variability however was closely related to the mean HbA1c of all patients ( $r = 0.55$ ), which could simply reflect the well-known association between the total glucose exposure and the risk for developing microvascular complications. In addition, the same investigators [65] failed to find any association between the development of diabetic retinopathy and the quarterly 7-point glycaemic profiles recorded over 3 consecutive days. This controversy did not therefore help to clarify the relationship between diabetic microvascular complications and long-term glycaemic variability.

### **The 2015 meta-analysis**

A few years later, Gorst et al [31] published a systematic review and meta-analysis involving 7 and 13 studies conducted in type 1 and type 2 diabetes, respectively. The authors found that a higher HbA1c variability was associated with an increased risk of cardiovascular events in both type 1 (risk ratio = 1.98 [CI95% = 1.39 - 2.82] and type 2 diabetes (risk ratio = 1.27 [1.15 - 1.40]). 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.

### **Recent studies**

Taking into account the limitations of the previous aforementioned studies, it is important to report the findings from several recent studies with the aim to decipher whether there exists or not an association between the long-term glycaemic variability and cardiovascular diseases in diabetes [33,47,63,66]. Even though these latest studies are essentially similar in design to the post hoc analysis of earlier trials, they have the advantage to have been conducted prospectively in highly selected and large populations with rigorous inclusion and exclusion criteria, over prolonged periods

of follow-up. As reported below these studies can be stratified according to their perceived degree of relevance.

a) The least relevant studies

- The post hoc analysis of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) [63] that was conducted over a median follow-up of 5 years in a large cohort of 4,892 individuals with and without diabetes tested the risk for developing cardiovascular events against the visit-to-visit variability of fasting blood glucose (FBG) when divided into quartiles with the lowest serving as reference. Glycaemic variability was derived from only three FBG measurements at 24-month intervals and further computed as 4 metrics: the SD, %CV, VIM and the ASG (Average Successive Variability). In the population considered as a whole, the participants in the highest quartile of glucose variability 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 mortality, but not cardiovascular events. One can only wonder how glycaemic variability can be meaningfully computed from so few FBG measurements and why the potential interplay between mean FBG and its variability was never evoked and tested, thus limiting the relevance of the reported findings.
- Analysis of the datasets of 54,803 subjects with diabetes ( $\geq 70$  years of age) included in The Health Improvement Network (THIN) study found that the overall mortality rate has a J-shaped distribution when plotted against mean HbA1c levels [47]. There was also a positive increasing relationship with HbA1c variability. Unfortunately, the types of diabetes were not accurately defined, the HbA1c data were also not collected in a controlled time-specified way, and finally the causes of deaths was never indicated. It is therefore difficult to know whether the enhanced mortality risk with the increasing long-term glycaemic variability score was due to cardiovascular events or other diseases and to agree with the authors' conclusions that long-term glycaemic variability (assessed from HbA1c variability) is an important risk factor in understanding the mortality risk in older people with diabetes.

b) The most relevant studies

Three studies published during the last two years 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 of these studies was based on the analysis of the English Clinical Practice Research Datalink (CPRD) database involving 58,832 people with type 2 diabetes, tested for HbA1c variability during the period from 2006 to 2009 and further followed for clinical

outcomes from 2010 to 2015. Having selected two groups of patients according to whether the average HbA1c was low ( $\leq 6.58$ ) or high ( $> 7.91\%$ ) the risk of all-cause mortality increased progressively in both groups with worsening HbA1c variability [66]. When the patients were separated into 2 groups by low or high HbA1c variability (coefficient of variation  $\leq 4.71\%$  or high  $> 11.40\%$ , respectively) the risk of all-cause mortality with worsening HbA1c became only significantly elevated when the mean HbA1c exceeded 8.88%. Such results suggest that focusing on both glycaemic stability and overall glycaemic control adds strength to the therapeutic strategy. However the results are limited due to the time lag of several years between the assessment of outcomes and the measurements of HbA1c metrics (mean and variability) during the run-in period of the study.

One of the post hoc analyses of the data provided by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [33] is of major interest when the 9,483 participants (4,728 and 4,755 in the standard and intensive therapy groups, respectively) were tested for HbA1c, HbA1c CV, VIM and ARV (Average Real Variability). The intensive and standard therapy groups were stratified into tertiles of mean HbA1c and the subgroups into tertiles of glycaemic variability (VIM). The cross tabulation analysis of mean HbA1c and long-term glycaemic variability (VIM of HbA1c) showed that the incident all-cause mortality increased with worsening mean HbA1c and with increasing VIM of HbA1c (figure 1). In addition another secondary analysis of the ACCORD trial [34] 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 higher risk of heart failure when compared with those in the lowest quintile (event rate = 6.2 vs 3.2%, respectively). Representing real-world evidence the LEAD (Louisiana Experiment Assessing Diabetes outcomes) cohort study involving 30,039 persons with type 2 diabetes over a mean follow-up period of 5.64 years demonstrated that the visit-to-visit HbA1c variability was an independent indicator of lower-extremity amputation. Multivariate adjusted Hazard Ratios for such adverse events increased from 1 to 1.88 across quartiles of HbA1c, CV ( $P$  trend = 0.012) [35].

Bringing all this data together, it seems that the long-term glycaemic variability (HbA1c) is strongly associated with the risk of adverse clinical outcomes in persons with diabetes. However, the debate continues as no one can confirm that this metrics of dysglycaemia is a causative factor for either cardiovascular events or all-cause mortality rather a simple marker of unsatisfactory adherence to lifestyle measures and/or pharmacologic treatments [67,68].



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

Short-term glycaemic variability is defined as rapid within or between-day upward and downward fluctuations of glucose concentrations [37]. In the 1970s several parameters such as the Mean Amplitude of Glycaemic Excursions (MAGE) [69] and the Mean Of Daily Differences (MODD)[70] were proposed to represent within and between-day glycaemic variability, respectively. At this time these parameters were mainly calculated from the self-monitoring of blood glucose because continuous glucose monitoring (CGM) was only available using intravenous blood sampling over limited periods of time in hospital settings specialized in the investigation of glucose homeostasis [71]. However, in the early years of the present century with the advent of subcutaneous glucose sensors CGM became available on an ambulatory basis and conducted over periods of several days up to two weeks with limited inconvenience. In parallel, there has been a profusion of new metrics [37,44,57,58,72,73], which unfortunately has created a greater degree of confusion [74] and lack of understanding by both healthcare professionals and patients. Therefore simple metrics should be recommended for the assessment of short-term glycaemic variability [37] as adopted at the International Conference on the Use of Continuous Glucose Monitoring [44]. The coefficient of variation for glucose was accepted 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 [45]. There is at present no evidence that short-term glycaemic variability is an independent risk factor for cardiovascular complications in diabetes [36]. However, absence of evidence is not synonymous with evidence of absence because a number of laboratory and clinical studies suggest that 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 [75,76] our seminal observation published in 2006 [28] represents a good start by demonstrating that the activation of oxidative stress, assessed from the urinary excretion rate of the free 8-isoprostaglandin F<sub>2</sub> $\alpha$  (8-isoPGF<sub>2</sub> $\alpha$ ) is strongly and positively correlated with glycaemic instability as assessed by the MAGE ( $r = 0.86$ ,  $P < 0.001$ ) in a limited group of poorly controlled people with type 2 diabetes (mean HbA<sub>1c</sub>  $\pm$  SD =  $9.67 \pm 1.30\%$ ) treated with oral antidiabetic agents alone . In addition, oscillating glucose concentrations in healthy subjects and non-insulin treated persons with type 2 diabetes were seen to be more deleterious to vascular endothelial function than sustained hyperglycaemia [77]. The relationship between the urinary excretion rate of 8-isoPGF<sub>2</sub> $\alpha$  and MAGE was not however evident in relatively well controlled type 1 diabetes (mean HbA<sub>1c</sub>  $\pm$  SD =

8.10 ± 0.90%) [78]. Two main explanatory factors can be proposed to explain these divergent results that raise several questions. Firstly, the discrepancies can be due to the differences observed in HbA1c levels [28,78]. Should this explanation be true, it implies that those exposed to chronic glucose exposure are more prone to suffer from adverse inflammatory and metabolic effects than those exposed to short-term glucose variability. A second more plausible explanation is based on the intrinsic inhibitory effect of exogenous insulin on inflammation, thrombosis and oxidative stress [79-81]. In insulin-requiring type 2 diabetes and type 1 diabetes we have observed that the urinary excretion rate of 8 isoPGF2 $\alpha$  was within the normal range observed in healthy non-diabetic controls [81]. In support to this latter hypothesis, a drastic reduction in the urinary excretion rate of 8-isoPGF2 $\alpha$  was observed when persons with type 2 diabetes treated with oral hypoglycaemic agents were converted to insulin treatments [81].

#### b) Lessons from interventional studies

A limited number of trials were conducted during the last past years and none led to convincing results. The FLAT-SUGAR trial [82] was designed to test whether a glucagon like peptide 1 receptor agonist-based regimen (exenatide, twice daily) added to basal insulin treatment in type 2 diabetes can reduce the short-term glycaemic variability and subsequently improve markers of cardiometabolic risk. In this study the exenatide arm was compared with participants treated only with a basal-bolus insulin regimen. No improvements occurred with the basal-bolus insulin regimen after 26 weeks of follow-up. The small reduction in short-term glycaemic variability seen 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-isoPGF2 $\alpha$ . As both arms in the FLAT-SUGAR trial were treated with insulin, the negative findings concerning these metrics are not surprising [83], thus providing additional support for the existence of an inhibitory action of insulin on inflammation, thrombosis and activation of oxidative stress [79-81]. The HEART2D study [84] also failed to find any difference in terms of cardiovascular outcomes when persons 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 future randomized controlled intervention trials aimed at testing the impact of short-term glycaemic variability on cardiovascular outcomes should therefore 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 [83].

#### c) Lessons from studies in critically ill patients

Hyperglycaemia has been reported to be associated with increased mortality in critically ill patients [85] whereas targeting euglycaemia [86] and glycaemic variability [87] appears to improve the prognosis. In a large population of individuals with (n = 942) and without diabetes (n = 3142) admitted to intensive care units for severe clinical conditions, mortality steadily increased with worsening mean glucose levels during their stay in hospital [88]. This relationship was only observed in individuals who were free of any glycaemic disorder prior to their hospital admission. However, when they were stratified by quartiles of mean glucose concentrations, there was a significant positive association between increments in glycaemic variability and mortality [88]. Surprisingly, a similar association was not observed in those with diabetes [88]. Consequently, such observational studies fail to define the optimal blood glucose targets needed to be achieved for the general populations hospitalized in intensive care units or even in high risk people with diabetes [89]. The use of CGM in intensive care can however contribute to a safer glycaemic profile [90], by minimizing the risk of hypoglycaemia through reducing glycaemic variability whilst benefitting from improved glycaemic control.

### ***3) Relationship between the short-term variability and hypoglycaemia***

The ACCORD study has established that attempting to achieve a too stringent glycaemic control in terms of mean HbA1c in type 2 diabetes results in increased frequency of hypoglycaemia [91]. Several years before, the DCCT Research group reported that the risk of severe hypoglycaemia in type 1 diabetes increased continuously with lower HbA1c values [92]. A few years later, with the emergence of the CGM technology, it rapidly appeared that excessive intra-day glucose fluctuations were associated with a higher risk of hypoglycaemia in those with diabetes and more specifically when the average HbA1c levels were near normal [93,94]. More recently, we used the CART (classification and regression tree) methodology to decipher whether short-term glycaemic variability is more significant than the mean daily glucose concentration in predicting the risk of unequivocal hypoglycaemia (glucose value < 3 mmol/L, i.e. 54 mg/dL) [46]. This analysis showed that short-term glycaemic variability when assessed as %CV and the mean daily glucose concentrations were not equivalent contributors to hypoglycaemia. The positive relationship between the time spent below 3 mmol/L and the %CV was more statistically significant than the inverse relationship observed when plotted against the mean daily glucose concentration [46] ([figures 2a and 2b](#)). Similar findings were observed soon afterwards by Ahmadi et al [95]. In addition, when the daily glucose value was < 7.8 mmol/L (140 mg/dL), a %CV below 34% is required for minimizing the risk of hypoglycaemia, very close to the cut-off value of 36% that separates stable from labile diabetes [45]. These observations indicate that persons with diabetes should therefore aim to reach a balance between chronic

ambient hyperglycaemia and glycaemic fluctuations in order to reduce both the long-term risk of developing diabetic complications and the acute risk of hypoglycaemia, respectively. Recent studies have demonstrated that parallel reductions in HbA1c, short-term glycaemic variability and frequency of hypoglycaemia can be simply obtained in CGM users with diabetes regardless of the modality of insulin delivery method [96-101]. The DIAMOND study [97] found that amongst participants with type 1 diabetes on multiple daily injections of insulin, CGM users achieved a significant improvement in HbA1c levels from 8.6 to 7.0 %, %CV from 42% to 38% ( $P < 0.001$ ) and reduced risk of hypoglycaemia irrespective of the selected alert threshold: 70 ( $P < 0.002$ ), 60 ( $P = 0.02$ ) or 50 mg/dL ( $P = 0.001$ ). Therefore, considering these studies as a whole, there is increasing evidence that the two objectives of limiting the risk of hypoglycaemic episodes by reducing short-term glycaemic variability and diminishing the mean glucose exposure to minimize the risk of long-term complications are not antinomic but complementary. In addition, this type of remark raises the question as to what extent does frequent hypoglycaemic episodes per se contribute to adverse clinical outcomes. It is well documented that severe hypoglycaemic episodes are associated with the risk of both acute cardiovascular events and chronic diabetes complications [102-107]. This relationship exists via their potential to promote cardiovascular events due to remains limited to surrogate outcomes [51] such as the induction of pro-arrhythmogenic cardiac disorders, endothelial dysfunction [108-110] and enhanced production of prothrombotic, proinflammatory or platelet proaggregant mediators or markers [111,112]. 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 [52,102]. Should we therefore not consider that hypoglycaemic episodes 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.***

Short-term glycaemic variability does not only consist of downward glucose fluctuations and hypoglycaemia, but is also the result from upward postprandial excursions. Therefore one of the remaining questions is to quantify the contribution of postprandial glucose increments to the overall glycaemic variability. In attempting to answer this question we have used the data from OPTIMAL trial involving 30 persons with type 2 diabetes [113]. This study was designed to compare the effects of the two DPP-4 inhibitors, sitagliptin and vildagliptin, added to ongoing treatment with metformin,

measuring several markers of glycaemic control after a period of 8 weeks. The post-hoc analysis of the changes from baseline showed that the  $\delta$ MAGE and  $\delta$ AUCpp (AUCpp = postprandial increments area above premeal glucose values) were strongly and positively correlated ( $R^2 = 0.48$ ,  $P < 0.0001$ ,  $n = 30$ ) (figure 3) [114]. As  $\delta$ AUCPP and  $\delta$ MAGE were plotted as explanatory (X) and dependent variables (Y), respectively, it can be calculated that in this regression situation 48%, i.e. approximately half of the overall short-term glycaemic variability is attributable to the amplitude of postprandial glucose excursions. Consequently, the upward and downward fluctuations around the mean daily glucose value contribute equally to the short-term glycaemic variability. A number of studies have demonstrated that treatments aimed at reducing postprandial glucose excursions with either short-acting GLP-1 Receptor Agonists (GLP-1 RAs) [82,84,115-117] or insulin analogs [118,119] reduce the overall glycaemic variability. In contrast, the short-term glucose variability remains essentially unchanged when implementing basal insulin regimens alone in type 2 diabetes, which only exert an overall downward translation of the 24-h glycaemic profiles without influencing the magnitude of postprandial glycaemic excursions [120,121]. Therefore reducing postprandial excursions appears to be one of the main recommendations to improve overall glycaemic variability and thereby reduce the activation of oxidative stress during postprandial periods [122].

### **Attempting to untangle the “Gordian knot”**

During the past few years, as highlighted in this review, an increasing number of publications have suggested or demonstrated the existence of a close interrelationship between glucose variability and diabetes complications. However, the relationships between these two variables were regarded more as being associated rather than linked by a cause and -effect relationship. This is mainly due to the reliance on retrospective analyses of clinical studies that were not specifically designed for deciphering whether long or short-term variability play an independent role in the development or progression of diabetes complications. Consequently, we still await specifically designed randomized interventional controlled trials where the effect of low- glycaemic variability would be tested against high-glycaemic variability, with everything else being equal, on the risk of diabetes related vascular complications. This would require an equivalent control of the ambient hyperglycaemia in both groups, extended over a prolonged 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 the known benefit of some antidiabetic drugs such as the GLP-1 RAs [123-127] or SGLT2 inhibitors [128-130] on cardiovascular diseases and insulin’s antithrombotic, anti-inflammatory or antioxidant effects [79-81]. The use of these agents in such trials should therefore be avoided or randomly distributed in equivalent proportions in the two

comparator groups [88]. When considering all these major difficulties, it seems almost impossible to conduct such a trial in a large number of participants who in addition should wear a CGM device over the entire period of follow-up. Consequently, the Gordian knot is far from being untangled. At present we are therefore only able to formulate a limited number of recommendations relating to classify the impact of glycaemic variability according to a decreasing graded scale of evidence. In an attempt to clarify the debate as far as possible, we have identified two categories of effects for the long-term, short-term variability and its composite risk factor, hypoglycaemia, that is: (i) limited to cardiometabolic markers and (ii) those extended to “hard” clinical outcomes. Reference to “medium/low supportive evidence” should be attributed to the first category because the results were obtained from observational studies. For the second category, the word “associations” should be employed because prospective interventional trials are lacking. However, several studies included in this latter category can be regarded as providing highly supportive evidence based on retrospective analysis of data collected over prolonged follow-up periods in well-conducted trials or observational studies. Considering all these remarks, the interrelationships are summarized in [figures 4a and 4b](#), whilst accepting that this representation is subject to further discussion in the future.

a) Evidence for cause and -effects of glycaemic variability on cardiometabolic markers with a medium/low grade of evidence are represented in figure 4a. Both the short-term glycaemic variability and hypoglycaemia appear to activate the production of cardiometabolic mediators or markers. By contrast, there is no similar evidence to suggest 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 strongly 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 independent 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) Currently there is medium/low supportive evidence that hypoglycaemia is associated with both short and long-term variability. The hypothesis of hypoglycaemia as an intermediary link in a catenary model from either short or long-term glucose variability to “hard” outcomes remains speculative. In addition there is no evidence available concerning the existence of likely interplay between short and long- term variability.

Finally, it is noteworthy that the role of the different components of glycaemic variability in the development of diabetic complications has been more carefully investigated during the last past few years than during the preceding decades, even though many aspects are still unclear. Targets have been clearly defined for short-term variability at %CV < 36% [44,45] and hypoglycaemia with a plasma glucose threshold of 70 mg/dL for any hypoglycaemic episode and 54 mg/dL for clinically significant hypoglycaemia [72]. Unfortunately such recommendations are missing for the long-term glycaemic variability (figure 5). 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, plasma glucose or multiple point glycaemic profiles [31].

In conclusion addressing the questions raised in the introduction of this review, all persons with diabetes should maintain the short-term glycaemic variability ideally below a %CV of 36 % [45,46] irrespective of the level of overall glucose exposure. This recommendation has a special relevance 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 respond as to whether HbA1c “cycling” is a non-acquired disorder or a consequence of an intermittent poor compliance to lifestyle changes and/or therapeutic measures [67].

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The authors have no conflict of interest relevant to this review

## 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 (adapted from reference 33)

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 46)

Figure 3: Relationship between changes in postprandial glucose increments ( $\delta$ AUCpp) and changes in short-term glycaemic variability ( $\delta$ MAGE) 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 114)

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: quaterly visit-to-visit changes in HbA1c, spot fasting plasma glucose at regular time intervals, monthly or quarterly multiple-point glycaemic profiles.



## References

- [1] Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J. Factors of risk in the development of coronary heart disease-six year follow-up experience: The Framingham Study. *Ann Intern Med* 1961;55:33-50.
- [2] Stamler J, Vaccaro O, Neaton JD, Wentworth D, for the Multiple Risk Factor Intervention Trial Research Group. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Interventional Trial. *Diabetes Care* 1993;16:434-44.
- [3] Mahmood SS, Levy D, Vasan RS, Wang TJ. The Framingham heart study and the epidemiology of cardiovascular diseases: a historical perspective. *Lancet* 2014;383:999-1008.
- [4] Gaede P, Vedel P, Larsen N, Jensen GVH, Parving H-H, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003;348:383-93.
- [5] Gaede P, Lund Andersen H, Parving H-H, Pedersen O. Effect of a multifactorial intervention on mortality in type 2 diabetes. *N Engl J Med* 2008;358:580-91.
- [6] Brown JD, Buscemi J, Milsom V, Malcom R, O'Neil PM. Effects on cardiovascular risk factor of weight losses limited to 5-10%. *Transl Behav Med* 2016;6:339-46.
- [7] Wing RR. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. *Arch Int Med* 2010;170:1566-75.
- [8] Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al, for the PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;368:1279-90.
- [9] Rosendorff C, Lackland DT, Allison M, Aronow WS, Black HR, Blumenthal RS, et al. Treatment of hypertension in patients with coronary heart disease. A scientific statement from the American Heart Association, American College of Cardiology and American Society of Hypertension. *Circulation* 2015;131:e435-70.
- [10] Adler AI, Stratton IM, Neil HAW, Matthews DR, Cull CA, Wright AD, et al, on behalf of the UK Prospective Diabetes Study Group. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *BMJ* 2000;321:412-9.
- [11] Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/APHa/ASPC/NLA/PCNA. Guidelines on the management of blood cholesterol. A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardio* 2019;73:e285-e350.
- [12] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidemias : lipid modification to reduce cardiovascular risk. The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *Eur Heart J* 2020;41:111-88.

- [13] Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000;321:405-11.
- [14] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008;359:1577-89.
- [15] The Diabetes Control and Complications Trial (DCCT) Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes. The DCCT/EDIC Study 30 year follow-up. *Diabetes Care* 2016;39:686-93.
- [16] Monnier L, Colette C, Schlienger J-L, Bauduceau B, Owens DR. Glucocentric risk factors for macrovascular complications in diabetes : glucose “legacy” and “variability”- what we see, know and try to comprehend. *Diabetes Metab* 2019;45:401-8.
- [17] Jeffery RW. Does weight cycling present a health risk? *Am J Clin Nutr* 1996;63 (suppl):452S-5S.
- [18] Field AE, Byers T, Hunter DJ, Laird NM, Manson JE, Williamson DF, et al. Weight cycling, weight gain, and risk of hypertension in women. *Am J Epidemiol* 1999;150:573-9.
- [19] Kakinami L, Knäuper B, Brunet J. Weight cycling is associated with adverse cardiometabolic markers in a cross-sectional representative US sample. *Epidemiol Community Health* 2020;74:662-7.
- [20] Mehta T, Smith DL, Muhammad J, Krista C. Impact of weight cycling on risk of morbidity and mortality. *Obes Rev* 2014;15:870-81.
- [21] Oh JT, Moon JH, Choi SH, Lim S, Park KS, Cho NH, et al. Body-weight fluctuations and incident diabetes mellitus, cardiovascular disease, and mortality: A 16-year prospective cohort study. *J Clin Endocrinol Metab* 2019;104:39-646.
- [22] Nam GE, Kim W, Han K, Lee C-W, Kwon Y, Han B, et al. Body weight variability and the risk of cardiovascular outcomes and mortality in patients with type 2 diabetes: A Nationwide Cohort Study. *Diabetes Care* 2020;43:2234-41.
- [23] Gosmanova EO, Mikkelsen MK, Molnar MZ, Lu JL, Yessayan LT, Kalantar-Zadeh K, et al. Association of systolic blood pressure variability with mortality, coronary heart disease, stroke, and renal disease. *J Am Coll Cardiol* 2016;68:1375-86.
- [24] Basson MD, Klug MG, Hostetter JE, Wynne J. Visit-to-visit variability of blood pressure is associated with hospitalization and mortality in an unselected adult population. *Am J Hypertens* 2018;31:1113-9.
- [25] Stevens SL, Wood S, Koshiaris C, Law K, Glasziou P, Stevens RJ, et al. Blood pressure variability and cardiovascular disease: systematic review and meta-analysis. *BMJ* 2016;354:i4098
- [26] Mehlum MH, Liestøl K, Kjeldsen SE. Blood pressure variability and risk of cardiovascular events and death in patients with hypertension and different baseline risks. *Eur Heart J* 2018; 39: 2243-51.
- [27] Nuyujukian DS, Koska J, Bahn G, Reaven PD, Zhou JJ, for the VADT investigators. Blood pressure variability and risk of heart failure in ACCORD and the VADT. *Diabetes Care* 2020;43:1471-8.
- [28] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol J-P, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006;295:1681-7.

- [29] Brownlee M, Hirsch IB. Glycemic variability: A hemoglobin A1c-independent risk factor for diabetes complications. *JAMA* 2006;295:1707-8.
- [30] Monnier L, Colette C, Owens D. The glycemic triumvirate and diabetic complications: Is the whole greater than the sum of its component parts? *Diabetes Res Clin Pract* 2012;95:303-11.
- [31] Gorst C, Kwok CS, Adam S, Buchan I, Kontopantelis E, Myint PK, et al. Long-term glycemic variability and risk of adverse outcomes: a systematic review and meta-analysis. *Diabetes Care* 2015;38:2354-69.
- [32] Hirakawa Y, Arima H, Zoungas S, Ninomiya T, Cooper M, Hamet P, et al. Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: The ADVANCE trial. *Diabetes Care* 2014;37:2359-65.
- [33] Scheng CS, Tian J, Miao Y, Cheng Y, Rang Y, Reaven PD, et al. Prognostic significance of long-term HbA1c variability for all-cause mortality in the ACCORD Trial. *Diabetes Care* 2020; 43:1185-90.
- [34] Segar MW, Patel KV, Vaduganathan M, Caughey MC, Butler J, Fonarow GC, et al. Association of long-term change and variability in glycemia with risk of incident heart failure among patients with type 2 diabetes: A secondary analysis of the ACCORD Trial. *Diabetes Care* 2020;43:1920-8.
- [35] Fan Y, Shen Y, Zhou J, Shi L, Nauman E, Katzmarzyk PT, et al. Visit-to-visit hemoglobin A1c variability is associated with the risk of lower-extremity amputation in patients with type 2 diabetes. *Diabetes Care* 2020;43:e178-80.
- [36] Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. *Lancet Diabetes Endocrinol* 2019;7:221-30.
- [37] Monnier L, Colette C, Owens DR. The application of simple metrics in the assessment of glycaemic variability. *Diabetes Metab* 2018;44:313-9.
- [38] Šoupal J, Škrha J Jr, Fajmon M, Horová E, Mráz M, Škrha J, et al. Glycemic variability is higher in type 1 diabetes patients with microvascular complications irrespective of glycemic control. *Diabetes Technol Ther* 2014;16:198-203.
- [39] Kilpatrick ES, Rigby AS, Atkins SL. A1c variability and the risk of microvascular complications in type 1 diabetes. *Diabetes Care* 2008;31:2198-202.
- [40] Zinman B, Marso SP, Poulter NR, Emerson SS, Pieber TR, Pratley RE, et al, on behalf of the DEVOTE Study Group. Day-to-day fasting glycaemic variability in DEVOTE: association with severe hypoglycaemia and cardiovascular outcomes (DEVOTE 2). *Diabetologia* 2018;61:48-57.
- [41] Pieber TR, Marso SP, Mc Guire DK, Zinman B, Poulter NR, Emerson SS, et al, on behalf of the DEVOTE Study Group. DEVOTE 3: temporal relationship between severe hypoglycaemia, cardiovascular outcomes and mortality. *Diabetologia* 2018;61:58-65.
- [42] Siegelar SE, Holleman F, Hockstra SB, DeVries JH. Glucose variability : does it matter ? *Endocr Rev* 2010;31:171-82.
- [43] Ceriello A. Glucose variability and diabetic complications: Is it time to treat? *Diabetes Care* 2020;43:1169-71.
- [44] Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care* 2017;40:1631-40.

- [45] Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N et al. Toward defining the threshold between low and high glucose variability in diabetes. *Diabetes Care* 2017;40:832-8.
- [46] Monnier L, Wojtusciszyn A, Molinari N, Colette C, Renard E, Owens D. Respective contributions of glycemic variability and mean daily glucose as predictors of hypoglycemia in type 1 diabetes : Are they equivalent? *Diabetes Care* 2020;43:821-7.
- [47] Forbes A, Murells T, Mulnier H, Sinclair AJ. Mean HbA1c, HbA1c variability and mortality in people with diabetes aged 70 years and older: a retrospective cohort study. *Lancet Diabetes Endocrinol* 2018;6:476-86.
- [48] International Hypoglycaemia Study Group. Hypoglycaemia, cardiovascular disease, and mortality in diabetes epidemiology, pathogenesis and management. *Lancet Diabetes Endocrinol* 2019;7:385-96.
- [49] Cryer PE. Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia. *Diabetes* 2014;63:2188-95.
- [50] Frier BM, Schernthaner G, Heller SR. Hypoglycemia and cardiovascular risks. *Diabetes Care* 2011;34 (Suppl 2):S132-7.
- [51] Connelly KA, Yan AT, Leiter LA, Bhatt DL, Verma S. Cardiovascular implications of hypoglycemia in diabetes mellitus. *Circulation* 2015;132:2345-50.
- [52] Zaccardi F, Ling S, Lawson C, Davies MJ, Khunti K. Severe hypoglycaemia and absolute risk of cause-specific mortality in individuals with type 2 diabetes: a UK primary care observational study. *Diabetologia* 2020;63:2129-39.
- [53] Hayward RA, Reaven PD, Wiitala XL, Bahn GD, Reda DJ, Ge L, et al, for the VADT Investigators. Follow-up of glycemic control and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2015;372:2197-206.
- [54] Monnier L, Bonnet F. Statistical and clinical significances: Are they equivalent? *Diabetes Metab* 2020;46:413-4.
- [55] Monnier L, Colette C, Bonnet F, Owens D. Number-Needed-to-Treat (NNT): Is it a necessary marker? *Diabetes Metab* 2020;46:262-4.
- [56] Funck-Brentano C. Indispensable but deceptive evidence-based medicine. *Diabetes Metab* 2020;46:415-22.
- [57] Rodbard D. Glucose variability: a review of clinical applications and research development. *Diabetes Technol Ther* 2018;20 (Suppl 2):S2-5-S2-15.
- [58] Kovatchev BP. Metrics for glycaemic control from HbA1c to continuous glucose monitoring. *Nat Rev Endocr* 2017;13:425-36.
- [59] Zar JH. Measure of dispersion and variability (chapter 4) In: *Biostatistical analysis* (Fourth Edition) Prentice Hall. Upper Saddle River, New Jersey 1999: pp 32-47.
- [60] Dolan E, O'Brien E. Blood pressure variability. *Hypertension* 2010;56:179-81.
- [61] Levitan EB, Kaciroti N, Oparil S, Julius S, Muntner P. Relationships between metrics of visit-to-visit variability of blood pressure. *J Hum Hypertens* 2013;27:589-93.
- [62] Muntner P, Whittle J, Lynch AL, Colantonio LD, Simpson LM, Einhorn PT, et al. Visit-to-visit variability of blood pressure and coronary heart disease, stroke, heart failure and mortality. *Ann Intern Med* 2015;163:329-38.
- [63] Echouffo-Tcheugui JB, Zhao S, Brock G, Matsouaka RA, Kline D, Joseph JJ. Visit-to-visit glycemic variability and risks of cardiovascular events and all-cause mortality: The ALLHAT Study. *Diabetes Care* 2019;42:486-93.

- [64] Monnier L, Colette C, Owens D. Comment on Echouffo-Tcheugui et al. Visit-to-visit glycemic variability and risks of cardiovascular events and all-cause mortality: The ALLHAT Study. *Diabetes Care* 2019;42:e104.
- [65] Kilpatrick ES, Rigby AS, Atkins SL. The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. *Diabetes Care* 2006;29:1486-90.
- [66] Crichley JA, Carey IA, Harris T, DeWilde S, Cook DG. Variability in glycated hemoglobin and risk of poor outcomes among people with type 2 diabetes in a large primary care cohort study. *Diabetes Care* 2019;42:2237-46.
- [67] Ceriello A, Rossi MC, De Cosmo S, Lucisano G, Pontremoli R, Fioretto P, et al, on behalf of the AMD-Annals Study Group. Overall quality of care predicts the variability of key risk factors for complications in type 2 diabetes: an observational longitudinal retrospective study. *Diabetes Care* 2019;42:514-9.
- [68] Edelman SV, Polonsky WH. Type 2 diabetes in the real world: the elusive nature of glycemic control. *Diabetes Care* 2017;40:1425-32.
- [69] Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes* 1970;19:644-55.
- [70] Molnar GD, Taylor WF, Ho MM. Day-to-day variations of continuously monitored glycaemia: a further measure of diabetic instability. *Diabetologia* 1972;8:342-8.
- [71] Service FJ, O'Brien PC, Rizza RA. Measurement of glucose control. *Diabetes Care* 1987;10:225-37.
- [72] Battelino R, Danne RM, Bergenstal SA, Amiel R, Beck R, Bister T, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the International Consensus on time in range. *Diabetes Care* 2019;42:1593-603.
- [73] Monnier L, Colette C, Owens D. Application of medium-term metrics for assessing glucose homeostasis: Usefulness, strengths and weaknesses. *Diabetes Metab* 2020 [Epub ahead of print] <https://doi.org/10.1016/diabet.2020.06.004>
- [74] Monnier L, Colette C, Owens D. Glucose variability : Do we have to revisit the profusion of definitions to avoid confusion? *Diabetes Metab* 2018;44:97-100.
- [75] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813-20.
- [76] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005;54:1615-25.
- [77] Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* 2008;57:1349-54.
- [78] Wentholt JM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. *Diabetologia* 2008;51:183-90.
- [79] Dandona P, Chaudhuri A, Ghanin H, Mohanty P. Insulin as an anti-inflammatory and antiatherogenic modulator. *J Am Coll Cardiol* 2009;53:S14-S20.
- [80] Wong VW, McLean MP, Boyages SC, Cheung NW. C-reactive protein levels following acute myocardial infarction: effect of insulin infusion and tight glycemic control . *Diabetes Care* 2004;27:2971-3.

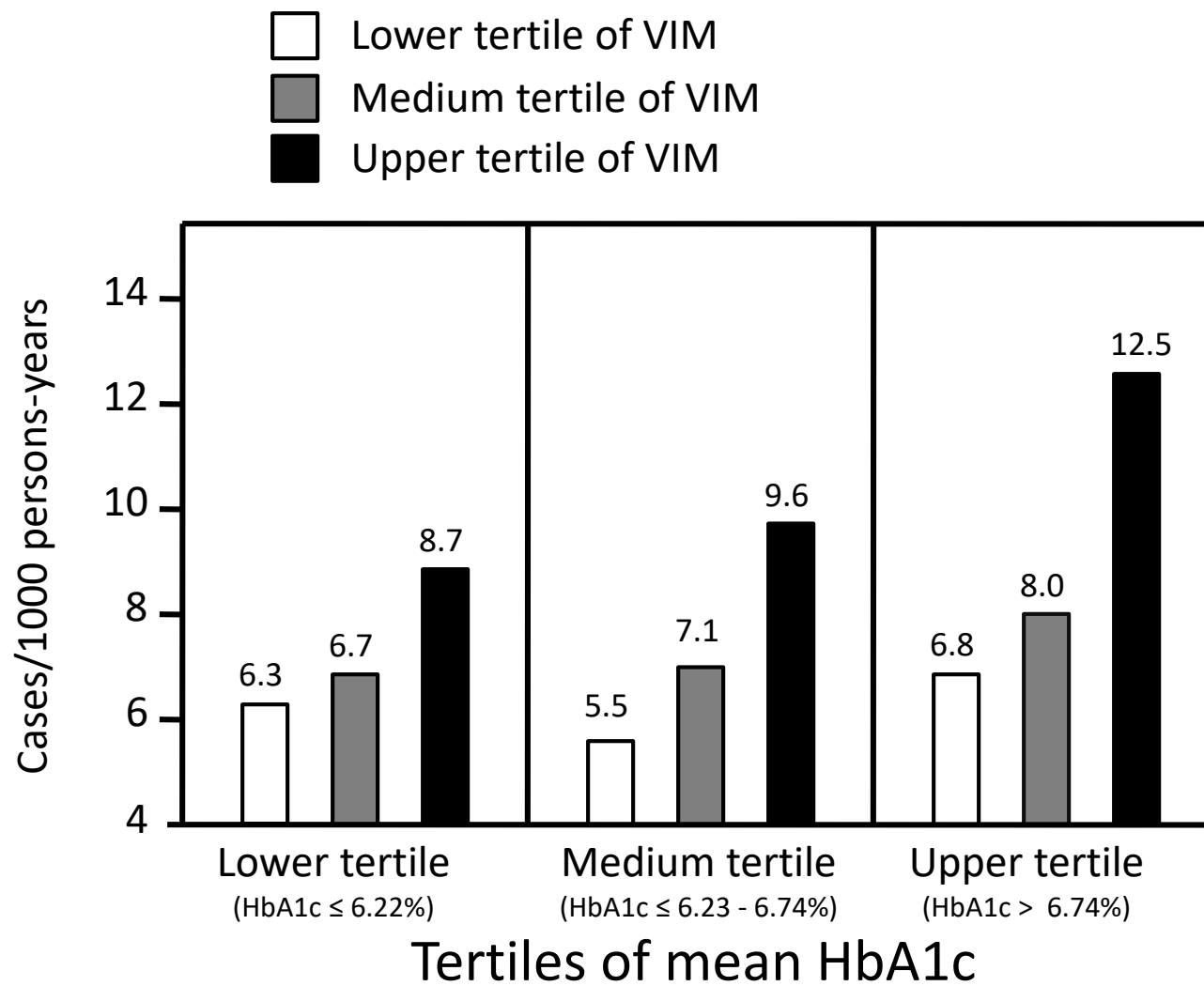
- [81] Monnier L, Colette C, Mas E, Michel F, Cristol J-P, Boegner C, et al. Regulation of oxidative stress by glycaemic control : evidence for an independent inhibitory effect of insulin therapy. *Diabetologia* 2010;53:562-71.
- [82] FLAT-SUGAR Trial Investigators. Glucose variability in a 26-week randomized comparison of mealtime treatment with rapid-acting insulin versus GLP-1 agonist in participants with type 2 diabetes at high cardiovascular risk. *Diabetes Care* 2016;39:973-81.
- [83] Monnier L, Colette C, Owens DR. Comment on the FLAT-SUGAR Trial Investigators. Glucose variability in a 26-week randomized comparison of mealtime treatment with rapid-acting insulin versus GLP-1 agonist in participants with type 2 diabetes at high cardiovascular risk. *Diabetes Care* 2016;39:e186-7.
- [84] Raz I, Wilson PWF, Strojek K, Kowalska I, Bozikov V, Gitt AK, et al. Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: The HEART2D trial. *Diabetes Care* 2009;32:381-6.
- [85] Krinsley JS. Association between hyperglycemia and increased mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc* 2003;78:1471-8.
- [86] Rady MY, Jonson DJ, Patel BM, Larson JS, Helmers RA. Influence of individual characteristics on outcome of glycemic control in intensive care unit patients with or without diabetes mellitus. *Mayo Clin Proc* 2005;80:1558-67.
- [87] Krinsley JS. Glycemic variability: a strong independent predictor of mortality in critically ill patients. *Critical Care Med* 2008;36:3008-13.
- [88] Krinsley JS. Glycemic variability and mortality in critically ill patients : The impact of diabetes. *J Diab Sci Technol* 2009;3:1292-301.
- [89] Krinsley JS. The long and winding road toward personalized glycemic control in the critically ill. *J Diabetes Sci Technol* 2018;12:26-32.
- [90] Preiser JC, Chase JG, Hovorka R, Joseph JI, Krinsley JS, De Block C, et al. Glucose control in the ICU : a continuing story. *J Diabetes Sci Technol* 2016;10:1372-81.
- [91] The Action to Control Cardiovascular Risk in Diabetes Study Group. Effect of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 2008;358:2545-59.
- [92] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin –dependent diabetes mellitus. *N Engl J Med* 1993;329:977-86.
- [93] Murata GH, Hoffman RM, Shah JH, Wendel CS, Duckworth WC. A probabilistic model for predicting hypoglycemia in type 2 diabetes mellitus. The diabetes outcomes in veterans study (DOVES). *Arch Int Med* 2004;164:1445-50.
- [94] Monnier L, Wojtusciszyn A, Colette C, Owens D. The contribution of glucose variability in asymptomatic hypoglycemia in persons with type 2 diabetes. *Diabetes Technol Ther* 2011;13:813-8.
- [95] Ahmadi SS, Westman K, Pivodic A, Ólafsdóttir AF, Dahlqvist S, Hirsch IB, et al. The association between HbA1c and time in hypoglycemia during CGM and self-monitoring of blood glucose in people with type 1 diabetes and multiple daily insulin injections: a randomized clinical trial (GOLD-4). *Diabetes Care* 2020;43:2017-24.
- [96] Lind M, Polonsky W, Hirsch IB, Heuse T, Bolinder J, Dahlqvist S, et al. Continuous glucose monitoring vs conventional therapy for glycemic control in adults with type 1 diabetes treated with multiple daily insulin injections: the GOLD randomized clinical trial. *JAMA* 2017;317:379-87.

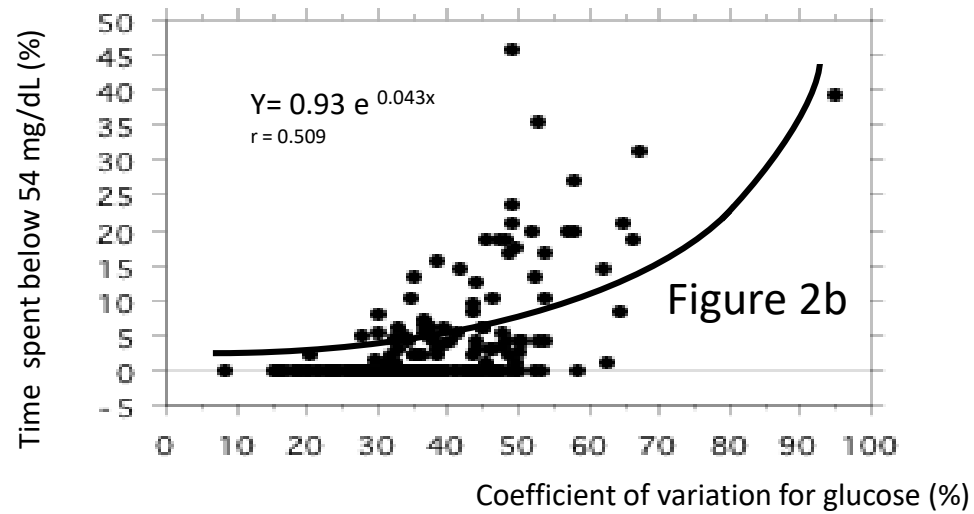
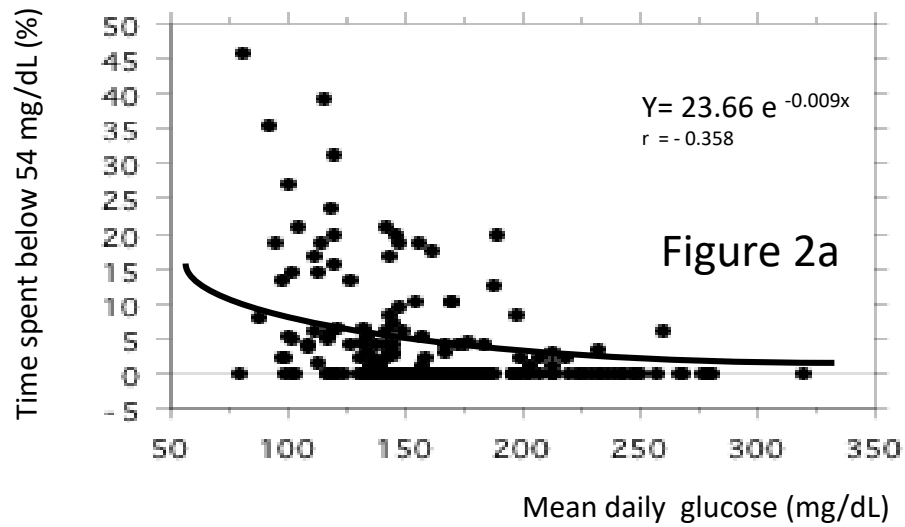
- [97] Beck RW, Riddleworth T, Ruedy K, Ahmann A, Bergenstal R, Haller S, et al. DIAMOND Study Group. Effect of continuous glucose monitoring on glycemic control in adults with type 1 diabetes using insulin injections: The DIAMOND randomized clinical trial. *JAMA* 2017;317:371-8.
- [98] Šoupal J, Petruzelková L, Flekac M, Pelci T, Matoulek M, Danková M, et al. Comparison of different treatment modalities for type 1 diabetes, including sensor-augmented insulin regimens, in 52 weeks of follow-up: a COMISAIR study. *Diabetes Technol Ther* 2016;18:532-8.
- [99] Laffel LM, Kanapka LG, Beck RW, Bergamo K, Clements MA, Criego A, et al. Effects of continuous glucose monitoring on glycemic control in adolescent and young adults with type 1 diabetes. A randomized clinical trial. *JAMA* 2020;323:2388-96.
- [100] Pratley RE, Kanapka LG, Rickels MR, Ahmann A, Aleppo G, Beck R, et al, for the Wireless Innovation for Seniors with Diabetes Mellitus (WISDM) Study Group. Effects of continuous glucose monitoring on hypoglycemia in older adults with type 1 diabetes. *JAMA* 2020;323:2397-406.
- [101] Šoupal J, Petruzelková L, Grunberger G, Hásková A, Flekac M, Matoulek M, et al. Glycemic outcomes in adults with T1D are impacted more by continuous glucose monitoring than by insulin delivery method: 3 years of follow-up from the COMISAIR Study. *Diabetes Care* 2020;43:37-43.
- [102] Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Biostat M, et al, for the ADVANCE Collaborative Group. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 2010;363:1410-1418
- [103] Yakubovich N, Gerstein HC. Serious cardiovascular outcomes in diabetes: the role of hypoglycemia. *Circulation* 2011;123:342-8.
- [104] Bedenis R, Price AH, Roberston CM, Morling JR, Frier BM, Strachan MW, et al. Association between severe hypoglycemia, adverse macrovascular events, and inflammation in the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2014;37:3301-8.
- [105] Goto A, Goto M, Terauchi Y, Yamaguchi N, Noda M. Association between severe hypoglycemia and cardiovascular disease in patients with type 2 diabetes. *J Am Heart Assoc* 2016;5:e002875.
- [106] Lee AK, Warren B, Lee CJ, Mc Evoy JW, Matsushita K, Huang ES, et al. The association of severe hypoglycemia with incident cardiovascular events and mortality in adults with type 2 diabetes. *Diabetes Care* 2018;41:104-11.
- [107] Yun J-S, Park Y-M, Han K, Cha S-A, Ahn Y-B, Ko S-Y. Severe hypoglycemia and the risk of cardiovascular disease and mortality in type 2 diabetes: a nationwide population-based cohort study. *Cardiovasc Diabetol* 2019;18:103-14.
- [108] Nordin C. The case for hypoglycaemia as a proarrhythmic event: basis and clinical evidence. *Diabetologia* 2010;53:1552-61.
- [109] Stahn A, Pistrosch F, Ganz X, Teige M, Koehler C, Bornstein S, et al. Relationship between hypoglycemic episodes and ventricular arrhythmias in patients with type 2 diabetes and cardiovascular diseases: silent hypoglycemia and silent arrhythmias. *Diabetes Care* 2014;37:516-20.
- [110] Pistrosch F, Gang X, Bornstein SR, Birkenfeld AL, Henkel E, Hanefeld M. Risk of risk factors for hypoglycemia and associated arrhythmias in patients with type 2 diabetes and

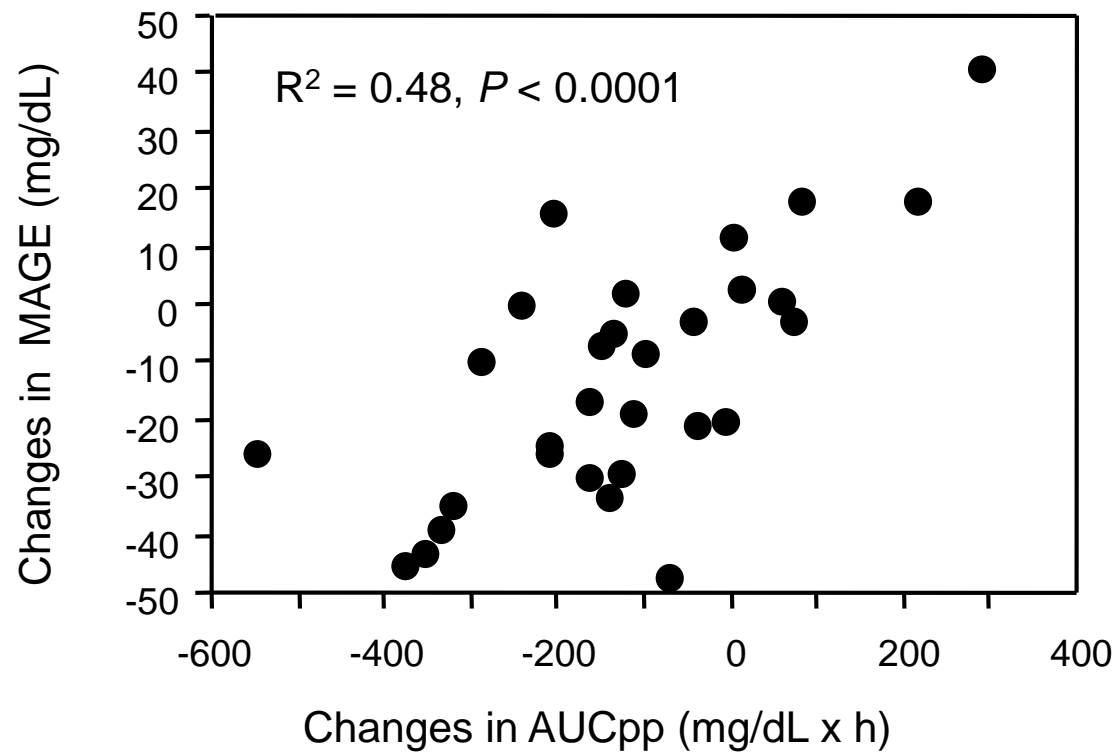
- cardiovascular disease: a cohort study under real-world conditions. *Acta Diabetol* 2015;52:889-95.
- [111] Monnier LH, Lachkar H, Richard J-L, Colette C, Borgel D, Orsetti A, et al. Plasma  $\beta$ -thromboglobulin response to insulin-induced hypoglycemia in type 1 diabetic patients. *Diabetes* 1984;33:907-9.
  - [112] Kahal H, Aburima A, Spurgeon R, Wraith KS, Rigby AS, Sathyapalan T, et al. Platelet function following induced hypoglycaemia in type 2 diabetes. *Diabetes Metab* 2018;44:431-6.
  - [113] Guerci B, Monnier L, Serusclat P, Petit C, Valensi P, Huet D, et al. Continuous glucose profiles with vildagliptin versus sitagliptin in add-on to metformin. Results from the randomized Optima study. *Diabetes Metab* 2012;38:359-66.
  - [114] Monnier L, Colette C, Dejager S, Owens DR. Mild dysglycemia in type 2 diabetes : to be neglected or not? *J Diabetes and its complications* 2015;29:451-8.
  - [115] Owens DR, Monnier L, Bolli GB. Differential effects of GLP1 receptor agonists on component of dysglycemia in individuals with type 2 diabetes mellitus. *Diabetes Metab* 2013;39:485-96.
  - [116] Diamant M, Nauck MA, Shaginian R, Malone JK, Cleall S, Reaney M, et al, for the 4B Study Group. Glucagon-like peptide 1 receptor agonist or bolus insulin with optimized basal insulin in type 2 diabetes. *Diabetes Care* 2014;37:2763-73.
  - [117] Meier JJ, Rosenstock J, Hincelin-Méry A, Roy-Duval C, Delfolie A, Coester H-V, et al. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care* 2015;38:1263-73.
  - [118] Home PD, Lindholm A, Hylleberg B, Round P, for the UK Insulin Aspart Study Group. Improved glycemic control with insulin aspart: a multicenter randomized double-blind cross over trial in type 1 diabetes. *Diabetes Care* 1998;21:1904-9.
  - [119] Owens DR, Bolli GB. The continuing quest for better subcutaneously administered prandial insulins: a review of recent developments and potential clinical implications. *Diabetes Obes Metab* 2020;22:743-54.
  - [120] Riddle MC, Rosenstock J, Gerich J, on behalf of the Insulin Glargine 4002 Study Investigators. The treat-to-target trial. Randomized addition of glargine or human NPH insulin to oral therapy of type 2 diabetic patients. *Diabetes Care* 2003;26:3080-6.
  - [121] Yki-Järvinen H, Kauppinen-Mäkelin R, Tiikkainen M, Vähätalo M, Virtamo H, Tulokas S, et al. Insulin glargine or NPH combined with metformin in type 2 diabetes: the LANMET study. *Diabetologia* 2006;49:442-51.
  - [122] Ceriello A, Quagliaro L, Catone B, Pascon R, Piazzola M, Bais B, et al. Role of hyperglycemia in nitrotyrosine postprandial generation. *Diabetes Care* 2002 ;25:1439-43.
  - [123] Marso SP, Bain SC, Consolli A, Eliaschewitz FG, Jodar E, Leiter LA, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2016;375:1834-44.
  - [124] Marso SP, Daniels GH, Brown-Frandsen E, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2016;375:311-22.



- [125] Gerstein HC, Colhoun HM, Dagenais GR, Diaz R, Lakshmanan M, Pais P, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind randomised placebo-controlled trial. *Lancet* 2019;394:121-30.
- [126] Hernandez AF, Green JB, Janmohamed S, d'Agostino RB, Granger CB, Jone NP, et al. Albiglutide and cardiovascular outcomes in patients with type 2 diabetes and cardiovascular disease (Harmony Outcomes): a double-blind randomised placebo-controlled trial. *Lancet* 2018;392:1519-29.
- [127] Cefalu WT, Kaul S, Gerstein HC, Holman RR, Zinman B, Skyler JS, et al. Cardiovascular outcomes trials in type 2 diabetes: where do we go from here? Reflections from a Diabetes Care editor's expert forum. *Diabetes Care* 2018;41:14-31.
- [128] Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al, for the EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes and mortality in type 2 diabetes. *N Engl J Med* 2015;373:2117-28.
- [129] Lupca BC, Inzucchi SE. Use of SGLT2 inhibitors in type 2 diabetes: weighing the risks and benefits. *Diabetologia* 2018;61:2118-25.
- [130] Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019;393:31-9.







Grade of evidence  
for causative effects  
on cardiometabolic  
markers/mediators

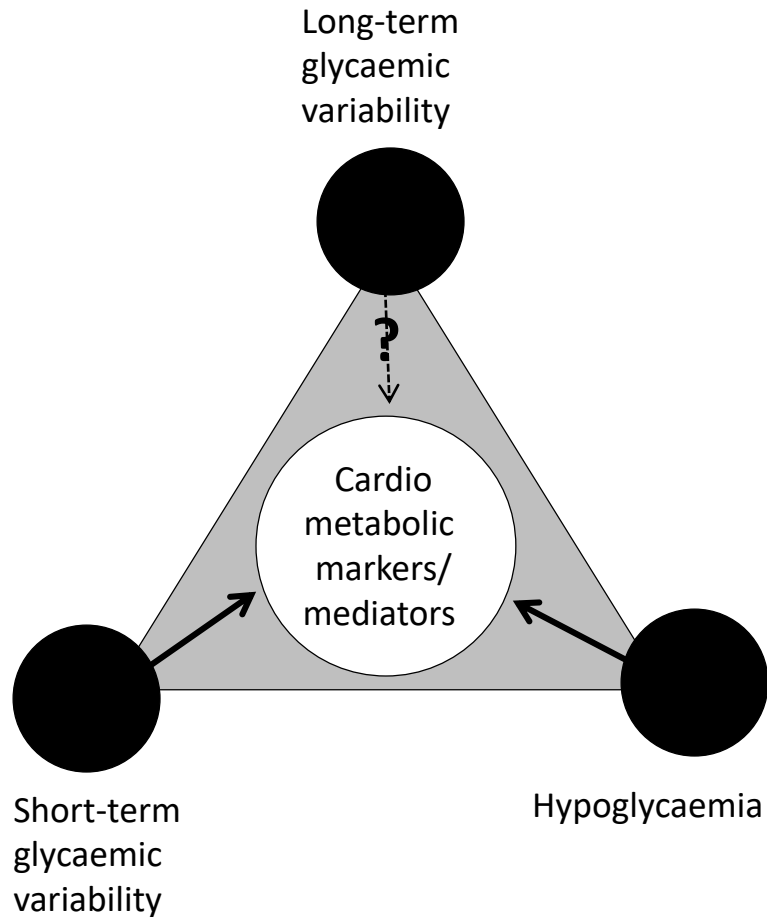


Figure 4a

Grade of evidence  
for association  
with « hard » adverse  
clinical outcomes

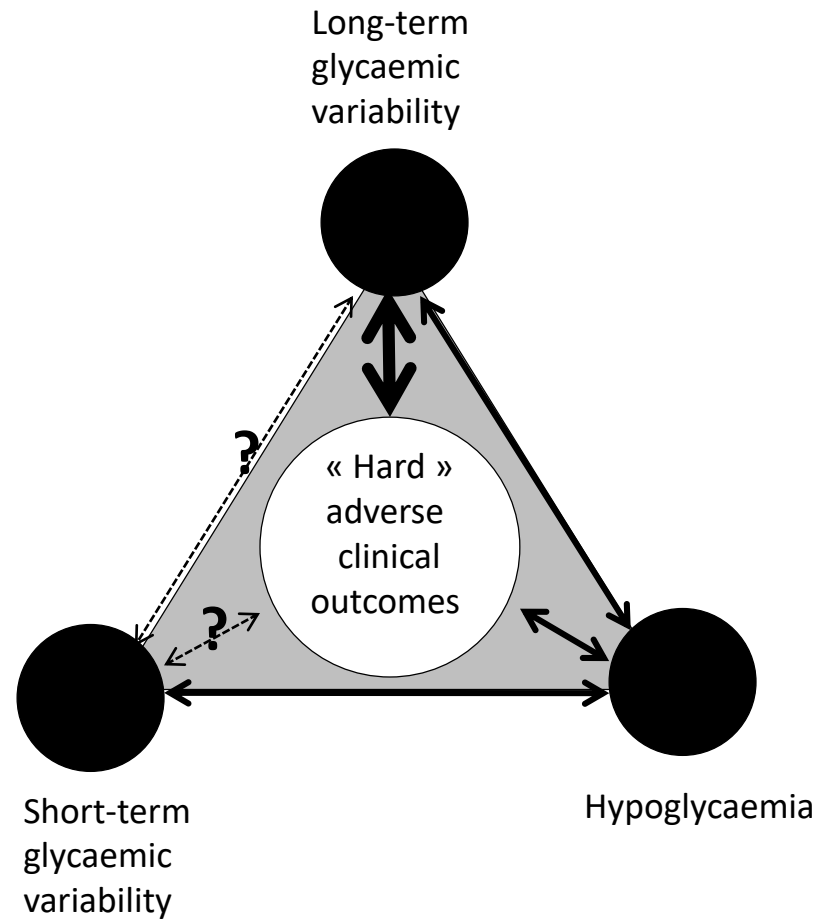


Figure 4b

Hypoglycaemia  
Alert thresholds : 70 and 54 mg/dL

