Huang Y et al. Endothelial Dysfunction and Platelet Hyperactivation in Diabetic Complications Induced by Glycemic Variability

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Introduction
Diabetes mellitus (DM) is the third most common chronic disease worldwide and a serious threat to human health and life [1]. DM-related macrovascular and microvascular complications, including coronary heart disease, cerebrovascular disease, heart failure, peripheral vascular disease, diabetic retinopathy (DR), neuropathy, and nephropathy, impair the quality of life and cause disability and premature death [2].

Fasting plasma glucose (FPG), postprandial glucose excursions, and hemoglobin A1c (HbA1c), described as the "glucose triad," are the main parameters used in monitoring patients with type 2 diabetes (T2D) [3]. Among elderly patients with T2D, all-cause mortality [4], including cardiovascular disease mortality [5], is mainly related to the variability or instability of fasting glycemia rather than its absolute values. In the Veteran Affairs Diabetes Trial, longitudinal variations in FPG were associated with all-cause mortality, even when accounting for standard measures of glucose control, as well as comorbidity and lifestyle factors [6]. Notably, many studies have found that HbA1c does not fully explain the risk of chronic complications of T2D. Therefore, other reliable and accurate monitoring parameters of diabetic complications need to be explored.

Good glucose control is one of the most effective means to prevent the complications of advanced DM [7]. Impaired glucose homeostasis is the main risk factor for cardiovascular disease [8]; thus, glycemic variability (GV) might be as important as the glucose triad [9]. Additionally, several studies have confirmed that patients with...
DM and fluctuant hyperglycemia have higher risks of chronic vascular complications than those with persistent hyperglycemia [10, 11]. Furthermore, greater degrees of glycemic fluctuation are associated with a higher incidence of complications and worse prognosis [12]. Thus, GV has become a research hotspot in the field of DM prevention and treatment.

Hyperglycemia fluctuation is an important factor that causes aggravation of DM-associated vascular complications. Vascular endothelial dysfunction is the initiating factor for the development of atherosclerosis and is an important pathophysiological basis for diabetic vascular disease. Platelet hyperactivation induced by thrombosis is also essential in the development of vascular events. Furthermore, both increased platelet reactivity and endothelial dysfunction are considered a "prothrombotic state" in DM [13]. This review aimed to provide a deeper understanding of the role and mechanism of GV-induced diabetic complications. Toward this goal, we focused on the relationship between endothelial dysfunction, platelet hyperactivation, and GV.

**Indicators of GV**

Glucose levels can be measured repeatedly in one day (within-day glucose variability) or during more days (between-day glucose variability) [14]. Another method is continual measurement of glucose levels using a continual glucose monitoring (CGM) system [15].

Dysglycemia in diabetes can be classified into two mechanisms: sustained chronic hyperglycemia and acute fluctuant fluctuations over a daily period [16, 17]. The former is integrated by HbA1c, which depends on both interprandial and postprandial hyperglycemia, and the percentage of each contributor is modulated by the degree of diabetic control [18]. There are many parameters for the clinical evaluation of GV [19], with the most common measures being the following (Table 1): (1) assessment of within-day blood glucose variability, including the mean amplitude of glycemic excursions (MAGE), standard deviation (SD) of all blood glucose measurements, and high/low blood glucose index (HBGI/LBGI); (2) assessment of day-to-day blood glucose variability, including the FPG coefficient of variation (CV), mean of daily difference (MODD), and average daily risk range (ADRR); (3) assessment of postprandial blood glucose fluctuation, such as the mean indices of meal excursions (MIME); and (4) special assessment for islet transplantation of type 1 DM (T1D), such as the lability index (LI).

The development of CGM technology greatly expands the ability to assess glycemic control throughout the day and has enabled research on the influence of acute blood glucose fluctuations in real life [36]. The 2017 Advanced Technologies & Treatments for Diabetes consensus conference [37] identified “time in ranges” as a metric of glycemic control that provides more actionable information than HbA1c alone. The metric includes three key CGM measurements: percentage of readings and time per day within target glucose range [TIR, 70–180 mg/dl (3.9–10.0 mmol/l)], time below target glucose range [TBR, 70 mg/dl (3.9 mmol/l)], and time above target glucose range [TAR, 180 mg/dl (10.0 mmol/l)] [38].

These variability parameters are important in selecting the optimal treatment strategies and estimating the risk of chronic DM complications. GV is closely related to diabetic complications and monitoring it could help in controlling or reducing the risk of complications. Therefore, the parameters of both short- and long-term GV should be further explored. Recently, numerous studies have supported the hypothesis that GV acts as an important determinant in both the genesis and development of diabetic vascular complications [39–44].

**GV and vascular complications of DM**

Diabetic vascular complications are conventionally classified as microvascular and macrovascular according to the size and location of the blood vessels involved [45]. Several studies have demonstrated that the presence of macro- and microvascular complications in patients with DM is related not only to chronic hyperglycemia represented by HbA1c but also to acute glycemic fluctuations [46, 47]. There is substantial evidence supporting that GV has drawn a great attention for its role in macro- and microvascular complications in patients with T1D or T2D [48].

**GV and microvascular disease of DM**

Microvascular disease, mainly including retinopathy, nephropathy, and neuropathy, is strongly associated with hyperglycemia. Recently, studies have shown a positive association between GV and microvascular complications of diabetes [49]. The present review suggests that increased levels of short-term glucose variability, particularly in FPG levels, may contribute to the development of microvascular complications in T2D, whereas the role of increased short-term glucose variability in the development of microvascular complications in T1D is less evident [50].

**Retinopathy**

The risk of development and progression of retinopathy has been linked to glycemic exposure in many studies from the Diabetes Control and Complications Trial in 1995 [51]. DR is currently the leading cause of blindness among working-aged persons in the developed world [52]. Many studies in the present literature indicate that short-term GV may contribute to the development or progression of DR in T2D, while long-term GV, represented by HbA1c, appears to play a more important role in retinopathy in patients with T1D or T2D [53]. One study of 415 DR patients with T1D in a tertiary referral centre suggested prevention and early detection of retinopathy in T1D patients, to take HbA1c variability into account when optimizing glycemic control [54].

**Nephropathy**

In a cross-sectional study of patients with T1D, higher glucose variability, as estimated by SD, CV, and MAGE, was found significantly more often in those with elevated albuminuria than in those with normal albuminuria, although their mean HbA1c was comparable [50]. Moreover, in vivo studies have revealed that in patients with T2D, GV results in chronic kidney disease characterized by progressive proteinuria, which ultimately leads to end-stage renal failure [55].

**Neuropathy**

Many forms of neuropathy can occur, including sensory, motor, and autonomic neuropathies, in the setting of diabetes after the exclusion of other causes. Several retrospective longitudinal studies on patients with T1D have demonstrated that glycemic fluctuations may contribute to diabetic peripheral neuropathy [56] and cardio-
vascular autonomic neuropathy [57]. Moreover, high levels of GV, assessed via CGM, appear to have even more deleterious effects than sustained hyperglycemia in the pathogenesis of cardiovascular autonomic neuropathy and other cardiovascular complications in patients with T2D [58, 59].

Both GV, as derived from visit-to-visit FPG measurements using CV, and HbA1c ≥ 53 mmol/mol were potent predictors of diabetic peripheral polyneuropathy (DPN) in patients with T2D. The associations among HbA1c, GV, and DPN suggest a linked pathophysiologic mechanism, which may play a crucial role in clinical risk assessments [60].

**GV and macrovascular disease of DM**

There are three major manifestations of macrovascular disease: coronary artery disease, cerebrovascular disease, and peripheral artery disease. In patients with T1D and T2D, the role of increased short-term GV in the development of both micro- and macrovascular complications is less evident [50].

**Coronary artery disease**

DM increases the risk of myocardial infarction (MI) more than any other risk factor aside from cigarette smoking. Coronary artery disease is the most common macrovascular complication recorded in
patients with DM [61]. Fluctuant hyperglycemia has adverse effects on blood vessels that lead to cardiovascular and peripheral vascular diseases. Patients with T2D complicated by coronary heart disease have higher GV than those without coronary heart disease [62]. In addition, increased visit-to-visit GV has been shown to predict mortality in patients with T2D and acute MI [63]. Previous studies have already correlated an increased GV to the occurrence of adverse events in patients with acute coronary syndromes undergoing percutaneous coronary revascularization (PCI) [64, 65].

Cerebrovascular disease
Stroke incidence is elevated in the diabetic population, claiming a high cost in terms of morbidity and mortality [66]. More severe glyemic fluctuations in patients with T2D are associated with a greater risk of cerebral infarction and worse prognoses [67].

Peripheral artery disease
The risk of developing peripheral artery disease (PAD) is increased two- to four-fold in patients with DM compared to those without [68]. One-fourth of patients with PAD demonstrate progression of symptoms over 5 years and a rate of amputation of around 4 %. Diabetes not only affects large-calibre peripheral vessels but distal arterioles as well [66]. A recent large, retrospective cohort study reported that HbA1c and GV, as estimated using FPG-CV, were risk factors for PAD aside from other conventional risk factors in persons with T2D [69].

Endothelial dysfunction in GV induces DM-associated complications
Endothelial dysfunction is defined as a disorder in the capacity of the endothelium to maintain vascular homeostasis [70]. It represents one of the most important determinants of coronary vascular disease [71]. Endothelial dysfunction could contribute to insulin resistance, potentially unifying the etiology of DM and coronary vascular disease [72]. Many studies have confirmed that fluctuant hyperglycemia is more likely to cause vascular endothelial dysfunction than persistent hyperglycemia [73]. Moreover, glucose fluctuation negatively influences endothelial function [74].

Endothelial dysfunction arising from a chronic hyperglycemic state is the result of increased oxidative stress and overproduction of reactive oxygen species (ROS), reduced nitric oxide (NO), and increased expression of adhesion molecules and inflammatory reactions (Fig. 1).

Oxidative stress plays a critical role in the pathogenesis of diabetic complications and several vascular diseases [75]. As stated in the unifying theory for DM complications proposed by Brownlee, oxidative stress response accompanied by sharp fluctuations in blood sugar is an important mechanism of vascular endothelial dysfunction [76]. The association between GV and oxidative stress has also been investigated using CGM. Monnier et al. [77] found that the average 24-hour urinary excretion rate of free 8-iso-prostaglandin (PG) F2α, a marker of oxidative stress, in 21 patients with T2D significantly correlated with MAGE, a marker of GV assessed via CGM, and with the area under the curve of the mean postprandial increment of glucose level above preprandial values. This suggests that glyemic fluctuations during postprandial periods are more likely to induce oxidative stress. In vitro studies and animal models have also substantiated these results.

Our study found that intermittent high glucose levels (5.56–25 mmol/every 24 h) induced oxidative stress injury with an increase in advanced oxidative protein products (AOPPs) and a decrease in total antioxidant capacity (T-AOC), which led to increased cellular apoptosis in human umbilical vein endothelial cells (HUVECs), compared with a constant high-glucose setting (25 mmol/l). Furthermore, the trend of these indices was verified in streptozotocin (STZ)-induced diabetic rats with fluctuating hyperglycemia treatment [78]. These effects were amplified during glucose fluctuations, consistent with previous observations [79, 80].

NO produced by nitric oxide synthases (NOS) is the smallest gaseous intercellular messenger involved in the modulation of several processes (e.g., blood flow and platelet aggregation control) and is essential for maintaining vascular homeostasis [81]. Aside from being a vasodilator, NO reduces vascular permeability and the synthesis of monocyte and lymphocyte adhesion molecules, which contribute to the reduction of tissue oxidation and inflammation, platelet aggregation, and thrombogenic factor activation. These processes, in turn, lead to the reduction of typical inflammatory processes induced by hyperglycemia [82]. Impaired endothelial NOS activity and enhanced ROS production in DM result in diminished NO bioavailability and vascular damage [83]. Therefore, NO is considered an important anti-atherogenic molecule that is necessary to contain diabetic endothelial alterations [84, 85].
Inflammation is widely considered a key etiological factor that plays a vital role in the development of diabetic complications [86]. Otsuka et al. injected glucose into the intraperitoneal space of Sprague-Dawley rats to cause a temporary increase in blood glucose. The results indicated that a transient increase in blood glucose could induce increased adhesion of monocytes, macrophages, and aortic endothelial cells more than sustained hyperglycemia, thus increasing the surface area of arteriosclerotic injury. Additionally, studies in HUVECs have also yielded similar results, further confirming that fluctuant hyperglycemia can significantly increase the levels of inflammatory indicators (interleukin-6, tumor necrosis factor-α) and expression of adhesion molecules (ICAM-1, VCAM-1, and E-selectin) [90–92]. In human circulation, fluctuant hyperglycemia could induce tumour necrosis factor-α production in vivo [93]. A previous study showed that high glucose altered the expression profile of cytokines and chemokines via specific signalling pathways and can increase monocyte-endothelial adhesion in monocytes [94]. Acute glucose fluctuation may cause significant oxidative stress and inflammation in rat aortic endothelial cells, increase the adhesion of monocytes to rat aortic endothelial cells, and elevate endothelial cell apoptosis, resulting in severe cardiovascular injury [95].

Platelet hyperactivation in GV-induced DM-associated complications

Increased coagulation, impaired fibrinolysis, and endothelial dysfunction result in a prothrombotic state for which platelet hyperreactivity is said to be an established contributing factor. Complications arise owing to these hyperactive platelets that play a vital role in the pathophysiology of thrombotic events [96]. Platelets could both trigger thrombus formation and release oxidative, mitogenic, and vasoconstrictive substances that induce the development of local vascular lesions [97]. Studies have shown that platelet aggregation is significantly enhanced and is in a hyperactive state in patients with DM, with subsequent increase in microcapillary embolization and accelerated local vascular lesions [98, 99]. Increased platelet activities, such as altered morphology and function, may play a vital role in the development of diabetic vascular complications [100]. A study found that increased levels of the mean platelet volume, plateletcrit (PCT), and platelet-large cell ratio (P-LCR) are positively associated with the occurrence of increased HbA1c, retinopathy, nephropathy, and neuropathy individually in patients with DM [101]. Chronic hyperglycemia has been established as the cause of platelet activation and platelet hyperactivity in patients with DM [102]. For example, the profound increase in urinary excretion of 11-dehydro-TXB2 in patients with T2D suggests that acute hyperglycemia induces increased platelet activation from high shear-stress conditions [103]. T2D is associated with a greater production of 8-isopGF2α, a stable compound of non-cyclooxygenase peroxidation of arachidonic acid inducing vasoconstriction and platelet activation [104]. Basili et al. [105] found that acute glucose fluctuations strongly correlated with urinary excretion of 8-isopGF2α, but no relationship was observed when urinary 8-isopGF2α excretion rates were plotted against main markers of sustained hyperglycemia (HbA1c and mean daily glucose concentrations). In our previous study, we explored platelet aggregation in HUVECs exposed to different GV media and healthy platelets and found that endothelial cells intermittently incubated with high glucose showed a more relevant increase in maximum platelet aggregation rate than the increase observed in the stable high-glucose condition [106].

The significant factors causing increased platelet reactivity in patients with DM are hyperglycemia and insulin resistance. Hyperglycemia can cause dysfunctional platelet adhesion, aggregation, and release through the following mechanisms (Fig. 2) [107, 108]. First, hyperglycemia is responsible for non-enzymatic glycation of proteins on the surface of the platelet, which decreases membrane fluidity and increases the propensity of platelets to become activated [109]. Next, the platelet activation signalling pathway is ultimately mediated by glycoprotein IIb/IIIa receptor (GPIIb/IIIa) platelet-fibrin interaction. Hyperglycemia leads to the release of larger platelets with more GPIb and GPIIIa receptors, thus increasing the aggregation baseline activation and thromboxane-forming capacity [110]. Third, both acute and chronic hyperglycemia induce increased protein kinase C (PKC) in vivo, a transduction pathway that triggers platelet activation [111]. Fourth, hyperglycemia promotes increased non-enzymatic glycation of circulating low-density lipoprotein, which may cause platelet dysfunction by increasing intracellular calcium concentration and platelet NO production [112, 113]. As an important second messenger, calcium participates in the regulation of a series of platelet functions, including morphological changes, secretion, aggregation, and thromboxane synthesis. Lastly, hyperglycemia induces the coagulation mechanism by increasing the release of prothrombotic molecules (e.g., tissue factor and von Willebrand factor [vWF]) while inhibiting fibrinolysis by increasing plasminogen activator inhibitor-1 (PAL-1) concentration [114, 115]. A high level of vWF in the circulation correlates with increased platelet activation, suggesting that acute, short-term hyperglycemia in T2D may precipitate vascular occlusions by facilitating platelet activation [116]. Additionally, mechanisms of platelet dysfunction in DM include up-regulation of platelet P2Y purinoceptor 12 signalling, increased generation of thrombin, increased production of thromboxane A2 from arachidonic acid metabolism, and accelerated platelet turnover [117].
Nowadays, several studies have demonstrated that some anti-diabetic agents also have antithrombotic effects. The potential benefit to platelets may be related to the normalization of glycemie control, but other additional direct antithrombotic and anti-inflammatory mechanisms may be involved. The modulation of platelet activation by antidiabetic drugs may mitigate the risk of thrombotic events and contribute to cardiovascular protection in patients with DM [118]. Metformin, recommended as first-line therapy for newly diagnosed T2D by the American Diabetes Association, is associated with decreased cardiovascular risk, such as significant reduction in cardiovascular endpoints (MI and stroke) or all-cause mortality [119] and decreased macrovascular complications (MI, stroke, peripheral vascular disease) in patients with DM already on insulin therapy [120].

**Interaction between endothelial dysfunction and platelet hyperactivation in vascular complications of DM**

Several studies have demonstrated that hyperglycemia is the main mediator of endothelial dysfunction and platelet hyperaggregation in DM, which contributes to the development and progression of vascular complications. Interestingly, both endothelial dysfunction and platelet hyperactivation also regulate hyperglycemia-induced diabetic complications (▶ Fig. 3). However, their influence on this process remains unclear and needs further investigation.

Oxidative stress is the first link in the interaction between endothelial dysfunction and platelet hyperactivation in vascular complications of DM. Superoxide may increase platelet reactivity by enhancing intraplatelet activity to release calcium after activation [121]. In addition, oxidative stress impairs endothelial function and reduces the production of NO, thus increasing platelet reactivity [122].

The second link of the interaction is vWF, which is a glycoprotein released into the circulation by secretion from endothelial cells. Studies have reported that an elevated level of vWF is associated with a higher risk of thrombotic cardiovascular events in patients with DM [123, 124]. Hu et al. [125] showed that hyperglycemia-induced repression of microRNA-24 increases vWF expression and secretion in both patients with DM and diabetic mouse models. When the endothelial layer is disrupted, vWF binds to exposed collagen and then anchors platelets to the subendothelium. In addition, vWF is also able to bind to GPIb-IX and IIb-IIIa platelet receptors, promoting platelet aggregation and causing platelet plug formation [126].

Advanced glycation end-products (AGEs) mediate the linkage between endothelial dysfunction and platelet hyperactivation. Previous data have indicated that increased AGE production under hyperglycemic conditions can induce decreased endothelial NOS expression and increased endothelin-1 expression and ROS through AGE-specific receptors, leading to endothelial dysfunction [127, 128]. This further results in impaired vasodilatory response to NO [129] and enhancement of platelet aggregation in vivo and in vitro [130]. Another mechanism linking AGEs and platelet hyperreactivity is the increased expression of CD36, CD62, and CD63 on the platelet sur-
face membrane [131, 132], which is associated with enhanced platelet reactivity in vitro as well as enhanced arterial thrombosis in vivo.

Conclusions

The long-term hyperglycemic state in patients with DM leads to the development and progression of microvascular and macrovascular complications of DM and increases their all-cause and cardiovascular mortality. Current research shows that GV leads to the development of vascular complications in patients with DM. Therefore, aside from FPG, postprandial blood glucose, and HbA1c, glycemic fluctuations should also be considered when formulating a blood glucose-lowering regimen. In addition, GV should be a target of clinical intervention. GV measurement has become more accurate with the development of new technologies (e.g., CGM systems) and provides directions for clinical and basic research. However, there is still no gold standard for measuring GV. Further, more studies on the effects of glycemic fluctuations on vascular complications in patients with DM should be conducted, with the reduction of glycemic fluctuations as a therapeutic target. This will help clarify the effect of GV on hard endpoints such as the development and progression of microvascular and cardiovascular events in patients. An increasing number of clinical and basic studies are investigating the molecular mechanism of vascular complications mediated by GV. It appears that endothelial dysfunction and platelet hyperactivation mediated by excessive oxidative stress play important roles, and in-depth research on molecular mechanisms related to GV is needed to obtain data for developing novel treatment regimens. Due to two mechanisms of blood glucose fluctuations mediating diabetes complications in our paper, the literature cited were mainly basic studies. So clinical studies were not mentioned, which was our limitation. However, our previous study focused on the relationship between blood glucose fluctuation and the prognosis of diseases, especially in critically ill patients [133]. The correlation between GV and macro- or micro-vascular complications of T1D or T2D as well as other critical diseases remains to be further explored.

Author Contributions

Conceptualization and funding acquisition: YH and JW; Writing of the original draft: YH and LY; Writing, review, and editing: JQ, MG, JW; Supervision: SL. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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