

## Interstitial versus Blood Equilibrium in Glucose Concentration and its Impact on Subcutaneous Continuous Glucose Monitoring Systems

Cosimo Scuffi

Scientist, Scientific and Technology Affairs Department, A. Menarini Diagnostics, Florence, Italy

### Abstract

The relationship between both interstitial and blood glucose remains a debated topic, on which there is still no consensus. The experimental evidence suggests that blood and interstitial fluid glucose levels are correlated by a kinetic equilibrium, which as a consequence has a time and magnitude gradient in glucose concentration between blood and interstitium. Furthermore, this equilibrium can be perturbed by several physiological effects (such as foreign body response, wound-healing effect, etc.), with a consequent reduction of interstitial fluid glucose versus blood glucose correlation. In the present study, the impact of operating in the interstitium on continuous glucose monitoring systems (CGMs) will be discussed in depth, both for the application of CGMs in the management of diabetes and in other critical areas, such as tight glycaemic control in critically ill patients.

### Keywords

Continuous glucose monitoring, lag time delay, interstitial fluid, interstitium, subcutaneous

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**Correspondence:** Cosimo Scuffi, A. Menarini Diagnostics, via Sette Santi 3, 50131 Florence, Italy. E: cscuffi@menarini.it

Continuous glucose monitoring (CGM) technology has become a fundamental tool in all those therapeutic applications where the measurement of the concentration and the rate of change of intracorporeal glucose are required, such as in the management of diabetes,<sup>1</sup> in the development of closed-loop systems ('artificial pancreas')<sup>2,3</sup> and tight glycaemic control in critically ill patients.<sup>4–6</sup>

If compared with the blood plasma glucose concentration (BG) measurements performed by means of either standard laboratory methods (SLB), point of care systems (POCTs) or self-monitoring of blood glucose systems (SMBGs), the main competitive advantage of CGMs lies in the capability of providing both absolute values and trends of glucose concentration. This enhances the capability to detect rapid hypo- and hyperglycaemia events (usually missed by discontinuous BG measurements), and allows the possibility of evaluating glycaemic variability parameters, which are promising markers for the prevention of diabetic complications and poor outcomes in critically ill patients.<sup>7–9</sup> However, there are still some concerns regarding the accuracy and reliability of the actual subcutaneous CGM systems for the application in the most critical areas, such as closed-loop system development<sup>10</sup> and tight glycaemic control in critically ill patients.<sup>11</sup>

The first and still most important field of CGM application is the management of diabetes. CGMs are utilised both in real-time (RT-CGM) for personal use as adjunctive device to SMBGs in the guidance and optimisation of the insulin delivery (particularly for those subjects under intensive insulin treatment and continuous subcutaneous insulin infusion),<sup>12–17</sup> and as a blind/retrospective professional glycaemic Holter for diabetes diagnosis, therapy adjustment<sup>18–22</sup> and control of gestational diabetes during pregnancy.<sup>23,24</sup>

Several studies demonstrated that the use of CGMs in the management of type 1 and 2 people with diabetes can decrease the occurrence of hypo/hyperglycaemia events,<sup>25–31</sup> lower glycaemic variability,<sup>28</sup> improve metabolic control and glycated haemoglobin (HbA<sub>1c</sub>) values<sup>1,16,17,28–33</sup> and enhance both treatment safety and quality of life of people with diabetes.<sup>34–36</sup>

In the last decade several generations of CGMs for diabetes management were launched on the market, mainly from Medtronic, Dexcom, Abbot, and A. Menarini Diagnostics. In most cases these devices were subcutaneous, minimally invasive, amperometric/enzymatic biosensor-based systems<sup>37–39</sup> and consequently provided an indirect evaluation of the plasma blood glucose level by means of a glucose concentration measurement in the extracellular interstitial fluid (IF). When using these devices, the sensor (needle-shaped electrode) or the sampling probe (in the case of microdialysis-based systems) needs to be inserted through the skin inside the subcutaneous fatty tissue in order to come into contact with the IF.<sup>34</sup>

Despite being in the early stages of the CGM development, a number of IF sampling and measurement methods have been considered (optical methods,<sup>40–44</sup> capillary ultrafiltration,<sup>45</sup> iontophoresis,<sup>46–52</sup> hypodermic needles,<sup>53,54</sup> ultrasound,<sup>46,55</sup> open-flow microperfusion<sup>56</sup> and impedance/electromagnetic spectroscopies).<sup>57,58</sup> CMGs that showed the highest accuracy and reliability performances are those based on one of two types of technology:<sup>59</sup> (1) transcutaneous (or needle-type) systems, where the amperometric biosensor is situated on the tip of a thin needle directly implanted in the subcutaneous tissue<sup>60,61</sup> or (2) microdialysis-based systems, in which the glucose is harvested from the IF, by means of a constant flow of saline buffer, which passes through a subcutaneously implanted microdialysis probe, and leads to a biosensor flowcell placed downstream.<sup>62</sup> All of these systems have been designed to operate in the subcutis, rather

than in the blood, due to its easy and safe accessibility, and for the possibility of frequent sensor replacement.<sup>63,64</sup> Given that the aim of CGMs is to guide the insulin delivery and therapy adjustments, which are all based on the plasma glucose concentration, the subcutaneous CGMs (SUB-CGMs) are calibrated using plasma glucose values (capillary or venous), and thus their accuracy evaluation is performed in comparison to BG values as references.<sup>65</sup>

Although the glucose concentration in the IF (IFG) demonstrated to have an high correlation with the corresponding plasma glucose value, it is nevertheless known that the IFG differs from BG both in time, generally presenting changes in delay in regard to the BG (physiological lag time), and in their absolute value of glucose concentration. In terms of the use of SUB-CGMs in diabetes management, if, on the one hand, the difference between BG and IFG is considered acceptable when the CGM data are used (and calibrated) retrospectively, on the other hand, this raises concerns about the impact of a significant physiological lag time in the capability of CGMs to support in RT the person with diabetes in his or her clinical decisions (such as if to inject insulin or not, or insulin bolus dose).<sup>66,67</sup> Moreover, in the most critical areas of potential application of CGMs (closed-loop systems, tight glycaemic control), the glucose measurement/sampling in the IF is actually considered a tremendous limit to the possibility of achieving a truly RT monitoring, and with no risk of uncorrelated response against blood compartment due to physiological condition of the patient.<sup>68,69</sup> For these reasons, in order to achieve tight glycaemic control in critically ill patients, an increasing number of companies shifted the selected sampling site from the subcutaneous tissue to the blood compartment by developing intravascular CGMs.<sup>59,70-77</sup>

Finally, improving the comprehension of BG versus IFG relationship and of its impact on the SUB-CGMs is a crucial step for their further optimisation, improved accuracy evaluation and for the understanding of their objective application limits.

## Experimental Evidence

Due to the increasing interest in CGM technology, the physiological relationship between IF and plasma in glucose concentration has been a widely debated topic in the recent years, and thus it has been investigated through a relevant number of studies, a summary of which is reported in *Table 1*. These evaluations were performed especially in terms of time lag between the changes in BG and in IFG, reporting values ranging from -16 to 25 minute.<sup>78,79</sup> Due to the lack of a well-recognised standard method for the IFG sampling and measurement, a significant boost to these studies was given by the microdialysis technique,<sup>80-82</sup> and thereafter by the availability of CGMs.

The outcomes of all the studies reported in *Table 1* lead to the assumption that IFG and BG are related by a 'time gradient', consisting in the delay (or lag time) between BG and IFG changes, and a 'magnitude gradient', corresponding to the difference between IF and plasma in the glucose concentration excursion amplitude and absolute value during the steady-states.<sup>83-85</sup> Furthermore, several physiological conditions, related to both the foreign body response (FBR) to the sensor implantation<sup>68</sup> and to the investigated subjects themselves,<sup>5,64,69</sup> were identified to significantly affect the correlation between IFG and BG.

## Time Gradient

With the time gradient it is intended the delay, or lag time, observed between the variation of the BG value (usually referring to the venous plasma) and the corresponding change in IFG. The term 'lag time' usually refers only

to the physiological lag, and thus only to those physiological processes regulating the glucose exchange between blood and interstitium. However, in several studies, this also included the instrumental lag, specific to the device used for the IFG sampling and measurement. The instrumental lag time also comprises a sampling lag, corresponding to the time needed for glucose transport from the IF to the biosensor and a computational lag, related to all the calculation algorithms applied to the recorded raw CGM signal before the measurement result is returned by the device (e.g. moving average over a period, noise filters, etc.)

The main and ongoing concerns related to the lag time are if it is either positive or negative, and thus if the IFG follows or precedes the BG, and if its value is constant or variable.<sup>86</sup> In the large majority of studies it was observed that the IFG delayed with respect to the BG, independently of whether the BG is rising, falling or reaching nadirs/peaks, with a lag time value that ranged between 5 and 25 minutes,<sup>66,87</sup> thus confirming a kinetic equilibrium based on a 'two-compartment' model of glucose diffusion from blood to IF.<sup>64</sup> Furthermore, the lag time presented both high inter-<sup>67,68,83</sup> and intra-subject variability (depending on the rising or falling phase of BG,<sup>88</sup> from the applied insulin or glucose infusion rate,<sup>89</sup> etc.)

In other studies,<sup>78,79,90</sup> the IFG was found to anticipate the BG decrease during insulin-induced hypoglycaemia. This was explained by increased glucose uptake from the IF by the surrounding cells, which lead IFG to drop prior to BG, as described by the 'push-pull' model.<sup>91</sup>

## Magnitude Gradient

Magnitude gradient generically means the difference in absolute glucose concentration between plasma and IF. This is related both to the glucose concentration value in the two compartments during the steady-states, and to the magnitude of the respective glucose concentration excursions.<sup>64,83,92</sup>

Studies reported conflicting results about this topic. In some studies,<sup>87,93-95</sup> involving mainly healthy subjects, the magnitude of the IFG and BG excursions and their values in their respective steady-states were found to be similar. However, a large group of studies agrees that both the glycaemic excursions and steady-state values of IFG and BG can show significant differences,<sup>64,84,85,96</sup> even maintaining a high correlation. Kulcu et al.<sup>83</sup> reported a relevant difference in the glucose excursion magnitude between IFG and BG at high glycaemic values during rapid rises of BG. Caplin et al.<sup>84</sup> observed that, following to falling phases of BG, the glucose concentration in the interstitium throughout steady-states was lower than that of plasma for prolonged periods of time in people with diabetes,<sup>69</sup> confirming what was previously observed in animal studies.<sup>91,97</sup>

## Physiological Conditions Affecting Blood Glucose and Interstitial Fluid Glucose Correlation

The kinetic equilibrium between BG and IFG, which allows an explanation of both the time and magnitude gradients, can be perturbed by the characteristics of the subcutaneous tissue, which represents a non-standard matrix in continuous change, depending on the physiological conditions of the subject.<sup>64,91,98,99</sup>

Considering the case of minimally invasive CGMs, the insertion of a probe/sensor in the subcutaneous tissue causes a trauma at the implantation site, because of the disruption of the tissue structure, thus leading to a FBR.<sup>39,100,101</sup> Despite the development of increasingly miniaturised probes/sensors, composed of materials with higher degrees of biocompatibility,<sup>68,102</sup> several local tissue reactions are likely to occur at the implantation site:

**Table 1: Summary of Interstitial Glucose versus Blood Glucose Average Lag Time, Estimated Values and Related Study Information**

First Author/Year of Publication/Reference	Estimated Lag Time (Minutes)	Study Population	Interstitial Fluid Sampling Method	Measuring Principle or CGM System (If Any)
Shichiri M, 1986 <sup>169</sup>	5	People with diabetes (n=5)	Transcutaneous sensor	Enzymatic biosensor
Matthews D, 1988 <sup>170</sup>	0	Anaesthetised rats (n=11), healthy subjects (n=10)	Transcutaneous sensor	Enzymatic biosensor
Aalders AL, 1991 <sup>171</sup>	Rise = 4 Fall = 8	People with diabetes (n=10), healthy subjects (n=6)	Microdialysis	Enzymatic biosensor
Meyeroff C, 1992 <sup>172</sup>	0–18	People with type 2 diabetes (n=7), healthy subjects (n=13)	Microdialysis	Enzymatic biosensor
Sternberg F, 1996 <sup>90</sup>	2–12	IT people with diabetes (n=40)	Microdialysis	Enzymatic biosensor
Thomé-Duret V, 1996 <sup>78</sup>	–16	Anaesthetised rats	Transcutaneous sensor	Enzymatic biosensor
Schmidtke DW, 1998 <sup>79</sup>	9–25	Anaesthetised rats	Transcutaneous sensor	Enzymatic biosensor
Tamada JA, 1999 <sup>48</sup>	18±10	People with type 1 and 2 diabetes (n=92)	Reverse iontophoresis	GlucoWatch (Cygnus)
Smith A, 1999 <sup>173</sup>	2–4	Healthy subjects (n=4)	Microporation	Fluorescein detection
Rebrin K, 2000 <sup>64</sup>	5–12	Anaesthetised dogs	Transcutaneous sensor	MiniMed CGMS (Medtronic)
Gross T, 2000 <sup>174</sup>	10	People with type 1 or 2 diabetes (n=135)	Transcutaneous sensor	MiniMed CGMS (Medtronic)
Aussedat B, 2000 <sup>91</sup>	5–10	Anaesthetised rats	Transcutaneous sensor	Enzymatic biosensor
Feldman B, 2003 <sup>146</sup>	5	People with type 1 diabetes (n=30)	Transcutaneous sensor	FreeStyle Navigator (Abbott)
Boyne MS, 2003 <sup>66</sup>	4–10	People with type 1 diabetes (n=14)	Transcutaneous sensor	MiniMed CGMS (Medtronic)
Kulcu E, 2003 <sup>83</sup>	5	IT people with type 1 or 2 diabetes (n=51)	Reverse iontophoresis	GlucoWatch (Cygnus)
Varalli M, 2003 <sup>175</sup>	2	People with diabetes, healthy subjects	Transcutaneous sensor	Glucoday (A. Menarini Diagnostics)
Steil GM, 2005 <sup>87</sup>	3–8	Insulin-induced hypoglycaemia in healthy subjects (n=10)	Transcutaneous sensor	MiniMed CGMS (Medtronic)
Weinstein RL, 2007 <sup>176</sup>	13	People with type 1 diabetes (n=58)	Transcutaneous sensor	FreeStyle Navigator (Abbott)
Wentholt IM, 2007 <sup>177</sup>	6	People with type 1 diabetes (n=13)	Microdialysis	Glucoday (A. Menarini Diagnostics)
Groenendaal W, 2008 <sup>145</sup>	1–3 (dermis) 5–10 (adipose tissue)	Insulin-induced hypoglycaemia in healthy subject (n=10)	Transcutaneous sensor	MiniMed CGMS (Medtronic)
Kamath A, 2009 <sup>178</sup>	6±1	IT people with diabetes (n=117)	Transcutaneous sensor	SEVEN (Dexcom)
Bailey T, 2009 <sup>179</sup>	8	IT people with type 1 or 2 diabetes (n=51)	Transcutaneous sensor	SEVEN PLUS (Dexcom)
Garg SK, 2009 <sup>180</sup>	5±3 (Dexcom) 10±3 (Abbott)	People with type 1 diabetes (n=14)	Transcutaneous sensor	SEVEN (Dexcom), FreeStyle Navigator (Abbott)
Kovatchev BP, 2009 <sup>164</sup>	13	People with type 1 diabetes (n=28)	Transcutaneous sensor	FreeStyle Navigator (Abbott)
Mazze RS, 2009 <sup>67</sup>	0–20 (Dexcom) 17–32 (Medtronic)	IT people with type 1 or 2 diabetes (n=24)	Transcutaneous sensor	Minimed Guardian RT (Medtronic), STS (Dexcom)
Valgimigli F, 2010 <sup>181</sup>	11	People with type 1 and 2 diabetes (n=12)	Microdialysis	GlucoMen Day (A. Menarini Diagnostics)
McGarraugh G, 2011 <sup>182</sup>	10	People with type 1 diabetes (n=47)	Transcutaneous sensor	FreeStyle Navigator (Abbott)
Ward WK, 2011 <sup>183</sup>	Rise = 9 Fall = 2	People with type 1 diabetes (n=7)	Transcutaneous sensor	SEVEN PLUS (Dexcom)
Keenan DB, 2012 <sup>184</sup>	8–12	People with type 1 and 2 diabetes (n=97)	Transcutaneous sensor	Enlite Sensor (Medtronic)

CGM = continuous glucose monitor; IT = insulin treated.

- Biofouling: aspecific protein adsorption on the foreign body surface (collagen and other proteins are secreted into the IF space due to transforming grow factor  $\beta 1$  and connective tissue growth factor),<sup>103–105</sup> with a consequent obstruction to the glucose diffusion across the IF–probe interface.<sup>66,106–109</sup>
- Wound-healing effect: the wound-healing cascade<sup>104,110</sup> can increase the vascularisation around the implanted probe, modifying the inter-compartment glucose equilibrium between plasma and IF.<sup>102</sup> The formation of well-vascularised granulation tissue near to the implantation site is likely to enhance glucose diffusion at the sensor–tissue interface<sup>111,112</sup> compared with avascular fibrous encapsulation.<sup>113</sup>
- Localised accumulation of inflammatory cells, macrophages, monocytes, mast cells and the release of cytokines and chemokines;<sup>104,110</sup> can lead to increased oxygen and glucose consumption near to the implantation site.<sup>102,114,115</sup>
- Fibrous encapsulation at sensor–tissue interface can alter the diffusional equilibrium.<sup>63,115–121</sup>
- Proteolytic enzymes and free radicals,<sup>118,122,123</sup> and local drops of pH

due to foreign body digestion attempts,<sup>124</sup> can damage to the sensor/probe surface.

- Cycles of bleeding and coagulation at the implantation site can tremendously interfere the sensor–probe functionality.<sup>90,125–128</sup>

Generally the main effect of these local reactions is transitory and limited to the few hours after the probe implantation, until a new physiological equilibrium at the tissue/probe interface is reached.<sup>129</sup> However, the occurrence of pressures, collisions or mechanical shocks on the implantation area can reactivate the local reactions and thus alter the equilibrium at the sensor–tissue interface, leading to another transitory period of lowered IFG versus BG correlation,<sup>130–132</sup> or even to a definitive failure of the CGM sensor. Furthermore, these phenomena are considered as possible causes of the sensors drift in signal, usually observed in the most of the enzyme-based subcutaneous CGMs, and which can make them progressively less sensitive and accurate over time.<sup>63,66,110,119,133</sup>

In addition to local reactions, other physiological conditions can further impact on the IFG versus BG correlation, such as those related

to critically ill patients (oedema, shock, hypotension, etc.),<sup>5,64</sup> to post-surgical situations,<sup>134-136</sup> drug administration,<sup>112</sup> altered blood perfusion in the sensor area,<sup>69</sup> bodily thermoregulation<sup>137,138</sup> and physical exercise.<sup>139,140</sup>

Referring to studies results reported in *Table 1*, such a high variability of outcomes indicates that both time and magnitude gradients are extremely case dependent, and that their presence/absence, variability and absolute values are related to the specific experimental conditions in which each study was performed:<sup>92</sup> IF sampling method used (microdialysis, transdermal, iontophoresis, etc.); IF sampling site (epidermis, dermis, adipose tissue); IFG measuring method (CGMs or SLB); tested population (animals/humans, healthy or type 1/type 2 people with diabetes); glycaemic control protocol (oral glucose tolerance test [OGGT], insulin/glucose clamp, insulin-induced hypoglycaemia, etc.); appropriate subtraction of the instrumental time lag (of the CGM device or of the sampling/measurement method used); effect of noise reduction and other compensation/elaboration algorithms, which can increase the instrumental lag that is then erroneously perceived as physiological lag time;<sup>86,141,142</sup> CGM system calibration method.

### Models Describing Interstitium/ Blood Glucose Equilibrium

Although a total consensus was not yet achieved on the BG/IFG relationship, a number of mathematical models for the BG versus IFG equilibrium description have been proposed. These models are based on the assumption of a free diffusion of glucose molecule between blood and interstitium, and in its uptake from the IF by the surrounding cells.<sup>64,91,143,144</sup>

Rebrin and Steil<sup>64</sup> proposed a ‘two-compartments’ model, which described IF and blood as two independent compartments (or pools), separated by a diffusional barrier through which the glucose is free to diffuse based on its concentration gradient<sup>86,144</sup> (see *Figure 1*). Moreover, the glucose is cleared from the IF by the surrounding cells, depending from IFG. This model provides an effective mathematical description of two important phenomena experimentally observed in several studies: the IFG follows the BG with a certain lag time; during the steady-states the glucose concentration in the two compartments can be significantly different. The only concern about this model is the assumption that both the diffusion across the IF/blood barrier and the clearance from the IF are related to BG and IFG values by constants ( $K_{12}$ ,  $K_{21}$  and  $K_{02}$  in the equation of *Figure 1*), while several evidences suggest that the dependence of these diffusion processes by BG and IFG is variable over the time.

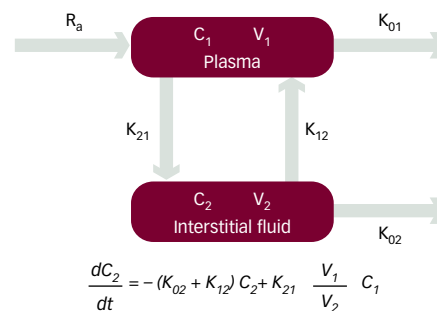
Another widely recognised model, based on the ‘push-pull’ effect, was proposed by Aussedat et al.<sup>91</sup> This approach hypothesised that during the rising of BG the lag time between BG and IF is caused by the glucose diffusion (push) from blood to IF, while during the falling period of BG the IFG decreases in advance with respect of BG, due to the insulin-induced uptake (pull) of glucose by the surrounding cells. This model allows explanation how in some particular conditions (e.g. insulin-induced hypoglycaemia) the drop of IFG can anticipate that of BG.<sup>78,79</sup>

The previous approaches were further refined by Groenendaal et al.,<sup>145</sup> reporting a model where a specific equilibrium between IFG and BG was described for each skin layer (epidermis, dermis, adipose tissue).

### Impact of Interstitium/Blood Differences on Continuous Glucose Monitoring Systems

The fact that SUB-CGMs provide an indirect BG evaluation by means of an IFG measurement entails a significant impact on the accuracy of these systems. These effects can be generically categorised into three types:

### Figure 1: Two-compartment Model Proposed by Rebrin and Steil<sup>64</sup>



Theoretical relation between the glucose concentration in plasma ( $C_1$ ) and in interstitial fluid ( $C_2$ ), based on diffusion ( $K_{12}$ ,  $K_{21}$ ) and clearance ( $K_{02}$ ) parameters.

pre-analysis effects, affecting the system prior of data collection (such as on calibration); intra-analysis effects, occurring during the measuring phase; and post-analysis effects, related to the CGMs accuracy evaluation.

### Pre-analysis Effects – Errors during the Calibration Phase

The calibration of a system that operates in a certain matrix, the IF, using measurement values collected in another matrix (venous/capillary blood) means that those differences existing between these matrixes will reduce the calibration quality, and thus the accuracy of the system.<sup>69</sup> This is in addition to the inherent error related to the calibration values itself, which can be significantly high if collected by means of SMBGs<sup>146</sup> (up to 20 % versus SLB).<sup>147</sup>

In order to minimise the error related to the BG versus IFG time gradient, all the manufacturers of CGMs require the system calibration to be performed using euglycaemic BG measurements collected during steady-states of the glycaemic value, where the resulting effect of the physiological lag time is negligible.<sup>146,148,149</sup>

Most of the CGMs are equipped with control algorithms capable of evaluating both the acceptability of the selected calibration values, based on the manufacturer’s criteria,<sup>150,151</sup> and the stability of the corresponding CGM signal,<sup>97</sup> prior that the calibration is definitively applied.

In order to overcome the BG versus IFG magnitude gradient, several two-point calibration methods have been developed.<sup>141,152</sup> These methods are based on the use of two independent BG calibration points, collected in distinct moments and required to be significantly different in the absolute glucose value (>30 mg/dl), for the estimation and calibration of the IFG excursion magnitude to those of the BG, for the sensor background current correction,<sup>63,111</sup> and also for reducing the impact of BG measurement system uncertainty.<sup>152</sup> Obviously these calibration methods are well applicable in the case of a retrospective analysis of the CGM data, while they are less effective and more error prone when applied in RT.

The physiological conditions affecting BG versus IFG correlation (see above), generally do not allow any calibration in the first period after probe implantation (run-in time) to be performed, and require a frequent system calibration in the first monitoring hours.

All the pre-analysis effects of the lag time can be mitigated in the retrospective calibration of the CGMs, where the availability of several BG references can allow the calibration versus both the time and magnitude gradient between BG and IFG to be optimised, by means of an algorithm-driven calibration points selection, and two-point or linear

regression methods.<sup>153,154</sup> For example, King et al.<sup>152</sup> demonstrated that a retrospective recalibration of the RT-calibrated CGM data allowed a relevant reduction of the mean absolute deviation during the steady euglycaemic state (from 20.9 down to 10.6 mg/dl). Similar results were showed by Facchinetti et al.,<sup>155</sup> where the mean absolute percentage error was reduced from 10.4 to 6.9 %. In the case of the RT calibration of CGMs the application of retrospective-wise selection of the calibration points is not applicable, and thus the impact on the calibration due to operate in the subcutaneous tissue cannot be significantly mitigated.

## Intra-analysis Effects – Lag Time and Loss of Interstitial Fluid Glucose/Blood Glucose Correlation during the Monitoring Session

The lag time between BG and IFG is the most recognised source of error in the CGMs. Rather than the presence of a delay, the real problem stems from delay variability. In several cases<sup>67,68,83,88,89</sup> the lag time was observed to be variable not only between different subjects, but also within the glycaemic profile of the same subject (e.g. depending from rising and falling phase of BG). Again, this problem is significantly mitigated when the CGM data are retrospectively used, since in this case the lag time (physiological and instrumental) can be evaluated and compensated prior to proceeding with the accuracy evaluation. Obviously, the higher the value and variability of the delay the lower will be the effectiveness of its evaluation and correction. On the contrary, the presence of the time lag, either constant or variable, cannot be eliminated in the RT CGMs application where glucose measurement will follow the BG value with a delay corresponding to the sum of physiological and instrumental lag, usually resulting in a value between 10 and 20 minutes. In this case an excessively high or inconsistent lag time could significantly decrease the effectiveness of the CGM device in controlling insulin delivery.<sup>66</sup> Another source of CGM inaccuracy during the monitoring session is related to the occurrence of those physiological conditions that can lower the BG and IFG correlation (see above).

The first strategy adopted for mitigating these kinds of CGM errors is the development of more and more biocompatible and miniaturised sensors,<sup>68,156</sup> in order to minimise the FBR and therefore the possibility of having local reactions after implantation.

A more-recent approach consists of redundant sensing, which is based on the use of multiple sensors contemporaneously, and in the combination of all the CGM signals collected in parallel.<sup>157</sup> The aim of this method is to detect individual aberrant (outlier) sensor values by means of the responses of the other sensors. Consequently, the crucial step in this approach is the processing method applied for combining the multiple data acquired.<sup>158</sup> The redundant sensing can also be 'orthogonal', that is, using sensors with different measuring principles, and thus are subjected to different interferences.<sup>159–161</sup>

On one hand this is a very promising way to increase the reliability of subcutaneous CGMs by correcting for gross errors, inconsistent data and all those evident system failures that are not expected to occur in all the implanted sensors. On the other hand, the capability of these approaches in enhancing the accuracy of CGMs has still to be demonstrated, especially against those physiological conditions affecting the BG versus IFG correlation.

Despite the current developments regarding biocompatibility, miniaturisation and redundant sensing, the CGM errors related to the loss of correlation between IF and blood cannot be avoided<sup>162</sup> either in the retrospective or in the RT CGM application, and can be detected only by the direct comparison with frequent BG reference measurements.

## Post-analysis Effects – Errors in the Accuracy Evaluation

Following the Performance Metrics for Continuous Interstitial Glucose Monitoring; Approved Guideline (POCT05-A),<sup>65</sup> the assessment of the CGMs' accuracy is actually performed using BG values as references. Consequently, each decrease in the IFG versus BG correlation is erroneously perceived as a CGM measurement error, and thus results in an apparent accuracy deterioration.<sup>163</sup> In order to take into account the contribution of the lag time, the guideline suggests that an evaluation, and possibly compensation, of the lag time prior to proceeding with the accuracy assessment. The suggested method is the Kovatchev's Poincaré Plot,<sup>164</sup> which consists of the calculation of Pearson's correlation coefficient R between CGM and BG reference data pairs, by applying increasing delays to the CGM signal. The applied delay corresponding to the highest R value is considered as the average lag time. The correction is then applied by shifting back in time the CGM data subtracting a delay equal to the calculated average lag time. Despite the fact this method is based on the assumption that the lag time is represented by a constant value (while the evidences suggest it to be variable), and that the correlation parameter used (R) is not ideal for the evaluation of data coming from different subjects with different glucose excursions,<sup>165</sup> it offers a good compromise between calculation simplicity and correction for the lag time error. Other lag time evaluation and correction methods, which account for a variable delay on the base of diffusional models, demonstrated the potentiality to further minimise the lag-time effect on the accuracy evaluation, but leading also to an increased calculation complexity.<sup>152,163</sup>

Another valuable improvement to CGMs' accuracy assessment was introduced by the Continuous Glucose Error Grid Analysis (CG-EGA),<sup>166</sup> which adapted Clarke's Error Grid Analysis,<sup>167</sup> intended for SMBGs, to the particular case of CGMs. The CG-EGA applies different point-accuracy acceptance criteria dependently from the rate of change of BG, and thus allows progressively higher tolerances in case of rapid glucose excursions. Although this method was not designed to directly correct for the lag time, considering that the lag time contribution to the CGMs errors is higher when rapid glucose fluctuations occur, the effect of the lag time is indirectly mitigated anyway.

## Conclusion

The relationship between interstitial and blood glucose is still a debated topic, on which a clear consensus has not yet been achieved. The experimental evidence suggest that BG and IFG are correlated by a kinetic equilibrium, which has as consequences a time and magnitude gradient in glucose concentration between blood and interstitium.<sup>83</sup> Furthermore, this equilibrium can be perturbed by several physiological effects (such as FBR, wound-healing effect, etc.), with a consequent reduction of IFG versus BG correlation.<sup>39,100,101</sup>

Regardless, the CGMs errors due to operate in the interstitium are considered acceptable in diabetes management. This is particularly valid when CGM data are retrospectively used, while in the RT application of CGMs the presence of a lag time still raises some concerns on their effectiveness in controlling insulin delivery.<sup>66,67</sup> Moreover, regarding the application in the most critical areas, such as tight glycaemic control on critically ill patients, the effects related to measuring in the subcutaneous tissue, instead of blood, are actually considered as a significant limit to SUB-CGMs utilisation, and thus suggest a shift in the development of CGMs, moving to CGM devices which operate directly in the intravascular compartment.<sup>59,70–77,168</sup> ■

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