A rat model of glycaemic variability. Reply to Zaccardi F, Pitocco D, Ghirlanda G [letter]

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Abbreviations

CONGAn	Continuous overall net glycaemic action
DM	Diabetic
GGC	Good glycaemic control
MAGE	Mean amplitude of glycaemic excursions
PGC	Poor glycaemic control

To the Editor: In their letter, Zaccardi and colleagues [1] express their opinion that the conclusion of our study [2], namely that ‘the oscillation of blood glucose level itself is a more important contributor to the loss of endothelial function in diabetes than the average level of mean blood glucose level per se’, is not completely supported by our data. The reason, as stated in their comments, is that we did not perform objective mathematical calculations of the various indices of glycaemic variability, such as mean amplitude of glycaemic excursions (MAGE), standard

deviation, continuous overall net glycaemic action (CONGAn), and others.

We are well aware of the various mathematical models developed for the estimation of blood glucose variability from data collected in the clinical setting mentioned by our esteemed colleagues [1]. These calculations are based on blood glucose concentrations in human patients: these patients have a circadian rhythm in their glucose metabolism and multiple postprandial glucose elevations over 24 h [3]. These models, with some modifications, can also be applied to our animal studies in order to compare the data from the control, diabetic (DM), diabetic/good glycaemic control (DM-GGC) and diabetic/poor glycaemic control (DM-PGC) animals. However, the circumstances in which our data were collected differ from those collected in the clinical setting in that: (1) the rats were fed ad libitum, making it impossible to examine postprandial glucose elevation; (2) the period of the glucose fluctuation was set to a 48 h cycle (as opposed to a 24 h cycle) in the DM-PGC group. The results, using appropriately modified formulas for calculations, show that standard deviation, MAGE and CONGAn (12 h) are, indeed, significantly higher in the DM-PGC than in the DM group (Table 1).

Thus, in our experimental animal model, the DM-PGC group had lower AUC values for plasma glucose levels over time and lower fructosamine levels than the DM group, while exhibiting markedly higher blood glucose variability. Strikingly, it was the DM-PGC group that had the highest degree of endothelial dysfunction and poly-(ADP-ribose) polymerase activation. Thus, we feel that the results shown in our original article [2], as well as the additional calculations contained in Table 1 here, continue to support our conclusion that it is the oscillation of blood glucose level itself, and not the average level of mean blood

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Table 1 Variables indicating the mean glucose concentration and glucose variability

Variable	Control	DM	DM-GGC	DM-PGC
Blood glucose AUC (mmol/l×h)	382.3±7.9	1,856±44.1 ^c	398.5±61.8 ^f	1,328±73.4 ^{c,f,h}
Fructosamine (μmol/l)	203.6±62.8	709.2±162.1 ^a	95.2±44.8 ^e	166.1±48.6 ^e
Blood glucose variability (mmol/l)				
SD (48 h)	0.7±0.1	7.5±1.0 ^c	4.1±1.0	12.7±1.3 ^{c,e,h}
MAGE (48 h)	1.4±0.2	17.9±2.1 ^c	10.4±1.7 ^{a,d}	35.7±3.1 ^{c,f,h}
CONGAn (6 h)	0.6±0.1	6.1±1.2 ^b	3.4±0.9	7.5±1.1 ^{c,g}
CONGAn (12 h)	0.4±0.2	3.3±1.2	3.3±0.9	8.2±1.7 ^{c,d,g}

^a $p \leq 0.5$, ^b $p \leq 0.01$, ^c $p \leq 0.001$ vs control; ^d $p \leq 0.5$, ^e $p \leq 0.01$, ^f $p \leq 0.001$ vs DM; ^g $p \leq 0.05$, ^h $p \leq 0.001$ vs DM-GGC

glucose level per se, that determines the development of endothelial dysfunction in our experimental model. We feel that this is the most straightforward explanation for the findings. According to the well-accepted overall principle of scientific parsimony (also known as Occam's razor), when there are a number of potential explanations for a given observation, the one with the least number of assumptions is preferred. Hence, we see no reason to invoke 'some other, unexplored variable' to explain the observed findings, contrary to the suggestion made by Zaccardi and colleagues.

Further work is needed to define whether these observations are relevant for humans, i.e. whether oscillating glucose contributes to the development of *human* macrovascular disease. Nevertheless, it is noteworthy that our conclusion is also supported by a number of human studies examining the connection between blood glucose variability (postprandial glucose concentration, glucose 'peak' and glucose 'spike') and macrovascular disorders. Carotid intima-media thickness was correlated with post-meal glucose peak, independently from HbA_{1c} level [4]. Oscillating blood glucose level was shown to cause a more severe endothelial dysfunction, as measured by the flow-mediated dilation of brachial artery, than constant high glucose concentration: the endothelial dysfunction was associated with increased oxidative stress and was reduced by the administration of the antioxidant ascorbic acid [5, 6]. Although flow-mediated dilation of brachial artery is only a surrogate endpoint, it has a very high predictive value (95%) for coronary heart disease [7].

Duality of interest E. Horváth declares no duality of interest associated with this manuscript. C. Szabo is a stockholder of Inotek Pharmaceuticals (Lexington, MA, USA), a company involved in the development of poly(ADP-ribose) polymerase (PARP) inhibitors and antioxidants.

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