DOI:10.1111/j.1464-5491.2010.03156.x

Reply from Buscemi S *et al.* Glycaemic variability using continuous glucose monitoring and endothelial function in the metabolic syndrome and in Type 2 diabetes. Authors' reply

Diabet Med. 28, 127–128 (2011)

We thank Zaccardi *et al*. for their insightful comments. Our study agrees with clamp experiments performed by Ceriello *et al*. which demonstrate *in vivo* an influence of oscillating glucose levels on endothelial function, even in subjects without diabetes [1]. However, clamp studies cannot fully reproduce physiological phenomena that occur in humans, hence our data, despite demonstrating only an independent association between glycaemic variability and flow-mediated dilatation of the brachial artery, are an interesting contribution to the debate concerning the influence of [here obtained from 48 h continuous subcutaneous glucose monitoring to cardiovascular risk. Zaccardi *et al*., on the basis of the study by Ceriello *et al*. [1], suggest that the influence of oscillating glucose measurements on.flow mediated dilatation may be limited to the 6 h preceding this measurement and not to the entire period of continuous subcutaneous monitoring. We did not, however, explore changes of flow mediated dilatation associated with those of glycaemic variability. We performed flow mediated dilatation the day before or the day after 48 h continuous subcutaneous glucose monitoring, in the morning and in postabsorptive conditions, after an overnight 12 h fast. Although we concur with the reasoning provided by Zaccardi *et al*., the question concerning the changes of flow mediated dilatation in the 6 h following oscillating glucose is not relevant to the present study.

In the study of Ceriello *et al*., the flow mediated dilatation values are reduced by more than 100% after hyperglycaemic spikes [1], values that cannot be comparable to our postabsorptive morning values. As we discussed in the manuscript, it is also important to distinguish the acute effect of postprandial hyperglycaemia from that of glycaemic variability, which is probably independent of the average glucose concentrations. The flow mediated dilatation measured in the morning in postabsorptive fasting conditions is a stable measure that can predict cardiovascular risk [2]. In our experience, 48 h continuous subcutaneous monitoring glycaemic variability is also a stable measure in short or intermediate time frames, particularly in subjects without diabetes, which may inevitably suggest that glycaemic variability may have a prolonged/chronic rather than an acute influence on the endothelial function. Furthermore, Zaccardi *et al*. postulate a biological explanation of the association between carotid intima–media thickness and glycaemic variability at 48 h. The carotid intima media thickness does not fluctuate within a 48 h time period, but may change in weeks [3]. Yet 48 h continuous monitoring glycaemic variability is likely to predict the usual glycaemic variability that chronically influences the flow mediated dilatation and therefore the carotid intima media thickness.

Finally, the concern regarding the lack of correlation between the 48 h continuous glucose monitoring area under the curve of glycaemia and flow mediated dilatation raises an interesting point. While a significant correlation was reported in the Appendix of $r = -0.34$ and a $P < 0.005$, the relationship was not independent and no longer contributed to the relationship between 48 h continuous monitoring mean glycaemia and flow mediated dilatation. As Zaccardi *et al*. correctly reported, the area under the curve is given by the product $k \times M$ (M is the mean glycaemia), where k is a constant given by the duration of the continuous monitoring.

Considering that the duration of continuous monitoring varies from 46 to 50 h, the constant will be different for each subject, hence influencing the area under the curve, which explains why our analysis demonstrated that mean 48 h continuous subcutaneous glucose monitoring showed an improved prediction for flow mediated dilatation compared with the area under the curve. Varying the constant may indeed significantly influence the value of the area under the curve, but predominantly for technical as opposed to biological reasons. The mean glycaemia is influenced less by the duration of the continuous subcutaneous glucose monitoring, given the fact that it is obtained on the basis of at least 700–1000 values, as we reported. In fact, if we perform multiple stepwise linear regression analysis excluding the 48 h continuous monitoring mean glycaemia, the area under the curve has a significant independent influence on the flow mediated dilatation ($\beta = -0.98 \times 10^{-5}$; $P < 0.05$) compared with glycaemic variability being maintained at an even higher significance level ($\beta = -0{,}12$; $P < 0.001$).

We appreciate Dr Boucher's comments. The hypothesis that seasonal variations in vitamin D status may influence at least in part both endothelial function and glycaemic variability is convincing. Although the hypothesis is intriguing, conflicting data exist that confirm this relationship [4]. Although we did not directly measure vitamin D concentrations, as Dr Boucher has suggested, we considered the date of measurements as a surrogate of vitamin D status. We now report no significant seasonal contributions to both the flow mediated dilatation variance ($F = 0.60$; $P = 0.61$) and the 48 h continuous glucose monitoring glycaemic variability ($F = 0.99$; $P = 0.40$); however, a larger cohort is probably necessary to explore such seasonal changes. In addition, our cohort lived in Sicily, located in the southern part of Italy, which has a significant degree of sunlight year-round. Although seasonal fluctuations of vitamin D status occur in temperate/tropical climates [5,6], blood concentrations are generally higher than those present in populations residing in northerly latitudes [7].We do agree, though, that future studies should ascertain vitamin D status, adjusting for seasonal variation to ascertain glycaemic homeostasis measures.

Competing interests

Nothing to declare.

S. Buscemi, J. A. Batsis*† and S. Verga

*Dipartimento di Medicina Interna, Malattie Cardiovascolari e NefroUrologiche, Facoltà di Medicina, University of Palermo, Italy, *Section of General Internal Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA and †Dartmouth College, Hanover, NH, USA*

References

1 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**: 1349–1354.

2 Rossi R, Nuzzo A, Origliani G, Modena MG. Prognostic role of flow-mediated and cardiac risk factors in post-menopausal women. *J Am Coll Cardiol* 2008; **51**: 997–1002.

3 Koshiyama H, Shimono D, Kuwamura N, Minamikawa J, Nakamura Y. Inhibitory effect of pioglitazone on carotid arterial wall thickness in type 2 diabetes. *J Clin Endocrinol Metab* 2001; **86**: 3452–3456.

4 Gulseth HL, Gjeldstad IM, Tierney AC, Lovegrove J, Defoort C, Blaak EE *et al*. Serum vitamin D concentration does not predict insulin action or secretion in European subjects with the metabolic syndrome. *Diabetes Care* 2010; **33**: 923–925.

5 Kristal-Boneh E, Froom O, Harari G, Ribak J. Seasonal changes in calciotropic hormones in Israeli men. *Eur J Epidemiol* 1999; **15**: 237–244.

6 Carnevale V, Modoni S, Pileri M, Di Giorgio A, Chiodini I, Minisola S *et al*. Longitudinal evaluation of vitamin D status in healthy subjects from southern Italy: seasonal and gender differences. *Osteoporos Int* 2001; **12**: 1026–1030.

7 Melin A, Wilske J, Ringertz H, Saaf M. Seasonal variations in serum levels of 25-hydroxyvitamin D and parathyroid hormone but no detectable change in femoral neck bone density in an older population with regular outdoor exposure. *J Am Geriatr Soc* 2001; **49**: 1190–1196.