Letters to the editor

Is it important to consider how hyperinsulinaemic–euglycaemic clamp results are expressed?

Est-il important de prendre en compte le mode d’expression des résultats du clamp hyperinsulinémique euglycémique ?

Keywords: Insulin resistance; Methods; Euglycemic hyperinsulinemic clamp; Insulin; Glucose

Mots clés : Insulinorésistance ; Clamp euglycémique hyperinsulinémique ; Insuline ; Glucose ; Méthodes

The hyperinsulinaemic–euglycaemic (HIEG) clamp is the gold standard for the measurement of insulin sensitivity in experimental research [1–3]. However, the expression of clamp data differs from one study to another in the literature. Indeed, clamp data are commonly expressed as total glucose disposal normalized per kilogram body weight (mg/kg min; also known as ‘M’) or per kilogram fat-free mass (FFM; mg/kg FFM min) [2,3]. Although normalization of insulin infusion (insulin/m² body area) is commonly used, the M value can be corrected by steady-state plasma insulin concentration (SSPI) during the clamp to avoid misinterpretation due to interindividual variations of insulinemia obtained during the test. Therefore, total glucose disposal can be expressed as M (either per kg of body weight or FFM/I), with I representing SSPI [1–3]. However, the results can also be expressed as $SI_{clamp} = M/(G \times \Delta I)$, wherein $G = \text{steady-state plasma glucose level}$ and $\Delta I = \text{difference between fasting and steady-state plasma insulin levels}$ [3].

Nevertheless, introducing insulin levels into the measure may add other confounders, such as differences in the insulin used for the test and in the assays used for its measurement. In addition, SSPI variation during the clamp could arise due to differences in insulin clearance between subjects, which may have an impact when results are expressed as $M/I$. Insulin infusion rates classically differ among studies, ranging from 40 to 80 mU/m² min, while duration of the clamp can vary from 2 to 3 h, with rather different results obtained accordingly [4]. Therefore, all of these aspects need to be taken into account when interpreting and comparing clamp data.

As an example, in the clamp database from the Centre de recherche en nutrition humaine Rhône-Alpes (CRNHA), we studied data from 108 non-diabetic subjects (age: 19–69 years; 73 women, 35 men) with a wide range of body mass index (BMI; 17.3–45.8 kg/m²; BMI < 25 kg/m², n = 49; BMI > 30 kg/m², n = 31) who participated in different studies carried out between 1995 and 2005 [5,6]. FFM was evaluated by measuring bioimpedance, and a standardized HIEG clamp was performed for each subject. Briefly, after an overnight fast, all patients underwent a 3-h hyperinsulinaemic glucose clamp with an insulin infusion rate of 75 mU/m² min [5,6]. During the last 30 min of the clamp, insulin and glucose were measured to obtain values in steady state. Clamp results were then expressed as $M$ (mg/min/kg), $M_{FFM}$ (mg/min/kg FFM), $M/I$ and $M_{FFM}/I$, with $I = \text{insulin (mU/L)}$ measured at steady state (mean of three values during the last 30 min of the clamp). For each way of expressing clamp data, the lowest quintile of values defined the insulin-resistant subjects and, as it is the most frequently used value, we arbitrarily considered the $M$ value as reference. Of the 21 subjects classified as insulin-resistant using $M$ as the reference for clamp results, 19 (89%) were still classified as insulin-resistant when the results were expressed as $M_{FFM}$. On the other hand, when the results were expressed as $M/I$ and $M_{FFM}/I$, only 13 (62%) and 13 (62%) subjects, respectively, remained insulin-resistant.

FFM and SSPI are the factors usually used to normalize $M$ values. As most glucose uptake takes place in muscles and only a little in adipose tissue, a glucose infusion rate expressed as $M$, which refers to whole-body glucose disposal, could overestimate insulin resistance in obese subjects, whereas its expression as $M_{FFM}$ should be more representative of insulin-dependent glucose consumption by muscle and would also be less affected by the subjects’ BMI [2,3].

However, our results showed that clamp data expressed as either $M_{FFM}$ or $M$ did not significantly change the insulin-resistant classification of the subjects (Chi² = 0.52; $P = 0.47$), suggesting that both forms of expression were similar in terms of classification. On the other hand, the glucose-disposal rate increases in proportion to plasma insulin concentration [2]. Thus, by taking the interindividual variations in clamp insulin concentration into account, the $M$ value can be normalized by SSPI. However, our results also suggest that such normalization significantly affects classification of insulin-resistance status (Chi² = 7.56; $P < 0.01$). Indeed, relating glucose utilization to insulin levels during the clamp by calculating the ratio of either $M/I$ or $M_{FFM}/I$ enables normalization of insulin-dependent glucose utilization to some degree while, at the same, introducing insulin clearance as a component of insulin sensitivity, which is
difficult to quantify in a hyperinsulinaemic state [7]. Nevertheless, insulin clearance rate may be an important consideration particularly in subjects with liver steatosis and hepatic insulin resistance [8,9].

In conclusion, although the HIEG clamp is considered the gold standard method for quantifying insulin sensitivity, the ways in which clamp results are expressed that correspond with different aspects of insulin sensitivity are not consensual and cannot be easily ascertained. This could lead to diverse classifications of the insulin-resistant status of subjects despite identical glucose infusion rates during the test. Thus, even with HIEG tests using similar insulin doses and durations, the potential effects of the various methods used to normalize data should always be borne in mind before interpreting and comparing insulin sensitivity obtained with the gold-standard HIEG clamp test. This factor might also explain some of the discrepancies observed between studies in the literature.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

We thank the volunteers who took part in the different experimental protocols and the CRNH-RA team for their excellent technical assistance.

Funding source: R. Rabasa-Lhoret holds the J.-A. Desève Chair in clinical research and is a recipient of a scholarship from FRSEQ (Fonds de recherche en santé du Québec [Quebec Fund for Health Research]).

References