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Relationships between insulin sensitivity measured with the oral minimal model and blood rheology

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Abstract. In studies using the intravenous glucose tolerance test with minimal model analysis we reported that low insulin sensitivity (S_I) is associated with increased erythrocyte aggregability and plasma viscosity, that appeared to be markers of insulin resistance. Recently, development of modelling has made available a new approach of insulin sensitivity from oral glucose tolerance test data (oral minimal model). We aimed at determining in 111 subjects (51 men, 62 women, age 11–77 yr), insulin sensitivity with this approach together with blood viscosity parameters. With this approach the Myrenne indexes of red cell aggregation were negatively correlated to S_I (M; $r = -0.456$; $p = 0.0007$; M1; $r = -0.397$; $p = 0.004$) while plasma viscosity was not. Correlations with fasting insulin levels (Ib) were weaker (M; $r = 0.2711$; $p = 0.05$; M1; $r = 0.373$; $p = 0.007$). Accordingly, a stepwise regression analysis selects M as the best correlate of SI and M1 as the best correlate of Ib. With this approach plasma viscosity does not exhibit any clear relationship with SI. This study supports the concept that RBC hyperaggregability is the prominent hemorheologic symptom of insulin resistance.

Keywords: Hemorheology, red cell aggregability, insulin resistance, insulin sensitivity, plasma viscosity, minimal model

1. Introduction

Insulin resistance is associated with a mild hyperviscosity syndrome [8, 11, 22–25, 31], which is more closely related to insulin resistance than to the clinical scoring of the metabolic syndrome [2, 3, 7]. In studies using the intravenous glucose tolerance test with minimal model analysis we reported that low insulin sensitivity (S_I) is associated with increased erythrocyte aggregability and plasma viscosity, that appeared to be markers of insulin resistance [26]. Recently, development of modelling has provided an increasing evidence that oral glucose tolerance tests (OGTT) and meal tolerance tests (MTT) are as powerful as intravenous glucose tolerance tests (IVGTTs) for assessing S_I, if they are analyzed with an appropriate mathematical algorithm [1, 15]. Several methods of calculation have been proposed for this

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Table 1
Characteristics of the 111 study subjects (51 men, 62 women)

Age (yr)	Weight (kg)	Height (m)	BMI (kg/m²)	FFM (Kg)	FM (Kg)	WHR
38.77 ± 1.44	79.70 ± 1.82	1.67 ± 0.01	28.67 ± 0.65	64.67 ± 3.10	11.72 ± 2.01	0.84 ± 0.03

BMI: body mass index; FFM: fat-free mass; FM: fat mass; WHR: waist-to-hip ratio.

analysis. Some of them rely upon sophisticated modelling, like Caumo's "oral minimal model" (OMM) [13] which is an extension to OGTT or MTT of the classical Bergman's minimal model analysis of IVGTT [6].

We thus aimed at determining in a sample of 111 subjects insulin sensitivity with this method together with blood viscosity parameters, in order to verify with another approach our preceding findings that increased erythrocyte aggregability and plasma viscosity are markers of insulin resistance.

2. Research design and methods

2.1. Subjects

We studied 111 subjects (51 men, 62 women, age 11–77 yr, body mass index ranging from 19.5 to 67.5 kg/m²). The mean systolic blood pressure was 132.44 ± 2.97 mmHg (ranging from 110 to 175) and diastolic blood pressure was 82.56 ± 2.14 mmHg (ranging from 70 to 120). Total cholesterol total was $2.24 \pm 0.05\,\mathrm{g\,l^{-1}}$ and triglycerides $1.26 \pm 0.12\,\mathrm{g\,l^{-1}}$. Mean clinical characteristics of subjects are shown on Table 1.

2.2. Measurements of insulin-sensitivity during the breakfast-test

Subjects had their breakfast [9] after clinical examination and blood sampling collection. It included bread (80 g), butter (10 g), jam (20 g), milk (80 mL), sugar (10 g) and instant coffee (2.5 g). Caloric intake was around 2070 kg (9.1% proteins; 27.5% lipids; 63.4% carbohydrates). Mean duration of food intake was 6 min. Blood samplings for plasma glucose and insulin were collected twice before breakfast and 15, 30, 60, 90, 120, 150, 180, 210 and 240 minutes after the beginning of the meal.

Caumo [13] extends Bergman's minimal model computation [6] to the analysis of meal test, such as this Standardized Hyperglucidic Breakfast (SHB), and provides an evaluation of S_I that can actually be calculated with a Microsoft Excel workbook according to the formulae published by its authors in their original paper [13, 17]. It is based on the analysis of changes in plasma glucose and insulin concentration measured after the SHB. S_I is given by the "oral minimal model" which is actually Bergman's one with simply another term called Ra_{OGTT} added to the first equation. Model equations are thus [13]:

$$\begin{cases} \dot{G}(t) = -[S_G + X(t)] \cdot G(t) + S_G \cdot G_b + \frac{R_{aogtt(a,t)}}{V} & G(0) = G_b \\ \dot{X}(t) = -p_2 \cdot X(t) + p_3 \cdot [I(t) - I_b] & X(0) = 0 \end{cases}$$

where G is plasma glucose concentration, I is plasma insulin concentration, suffix "b" denotes basal values, X is insulin action on glucose production and disposal, V is distribution volume, and S_G, p_2,

and p_3 are model parameters. Specially, S_G is the fractional (*i.e.*, per unit distribution volume) glucose effectiveness, which measures glucose ability per se to promote glucose disposal and inhibit glucose production; p_2 is the rate constant describing the dynamics of insulin action; p_3 is the parameter governing the magnitude of insulin action. Interestingly, these two equations can be simplified, allowing calculating S_I with a quite simple area under the curve formula:

$$S_{I(OGTT)} = \frac{f \cdot D_{OGTT} \cdot \frac{AUC[\Delta G(t)/G(t)]}{AUC[\Delta G(t)]} - GE \cdot AUC[\Delta G(t)/G(t)]}{AUC[\Delta I(t)]}$$

where G is plasma glucose concentration, ΔG and ΔI are glucose and insulin concentrations above basal, respectively, AUC denotes the area under the curve; GE (also called S_G) is glucose effectiveness ($min^{-1} \times 10^{-2}$); D_{OGTT} is the dose of ingested glucose per unit of body weight ($mg \cdot kg^{-1}$); and f is the fraction of ingested glucose that actually appears in the systemic circulation. When glucose falls below basal, a slightly different formula needs to be used (we refer to equation n°7 in Caumo et al. [13]). Calculations of S_I require insertion of values for S_G and f. Here we used the value of glucose effectiveness given by our previously validated formula $S_G = 2.921\ e^{-0.185(G_{60} - G_0)}$ [1]. Besides, as in Caumo's paper, the value for f is set as $f = 0.8$.

Caumo's model is the extension to oral glucose tolerance tests of Bergman's equations initially developped for the IVGTT. It has been shown to be suitable for calculating S_I during this hyperglucidic breakfast test [1, 16, 18], even in diabetic patients [10].

Control values with this technique are available on large databases and on 537 controls we defined the threshold for insulin resistance as the limit of lower quartile *i.e.*, $S_I < 3.27\ min^{-1} . \mu U^{-1} . mL^{-1}$.

2.3. Laboratory measurements

Viscometric measurements were done at very high shear rate ($1000\ s^{-1}$) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [19, 21]. The coefficient of variation of this method ranges between 0.6 and 0.8%. We measured with this device apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (45%) according to the equation of Quemada [27]. RBC aggregation was measured with laser backscattering (erythroagregometer SEFAM - AFFIBIO [14, 20]. Indices of RBC aggregation: (c): primary time of aggregation TA; (d) secondary time of aggregation d'agrégation TF; (e) index of structure Is; partial disaggregation threshold γD and total disaggregation threshold γS; (f) indices IA_{10} et IA_{60} which quantify the mean importance of aggregation at 10 and 60 sec. The updated guidelines for hemorheological laboratory techniques [5] were taken into account.

2.4. Statistics

Results are presented as mean ± SE of the mean. Before and after training, values were compared with the paired Student *t*-test after verification of the normality of distribution of differences between before and after values with the Kolmogorov-Smirnov test. Correlations were assessed with Pearson's procedure (least square fitting). A value of $p < 0.05$ was considered as significant. Linear correlations and stepwise correlation analyses were performed with the software "Sigmastat" from Jandel Scientific, San Jose, California, USA.

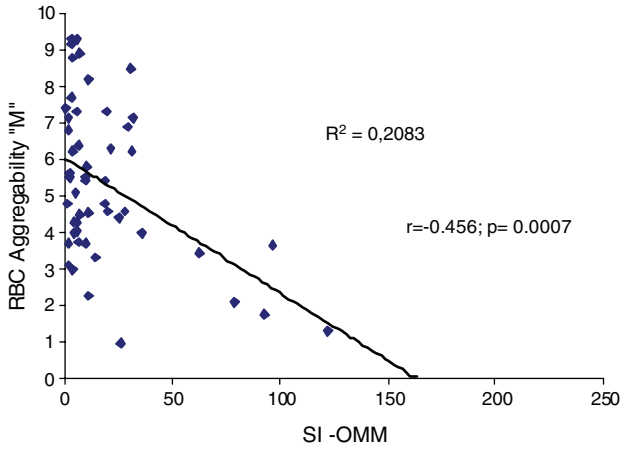

Fig. 1. Correlation between RBC aggregability index 'M' and insulin sensitivity.

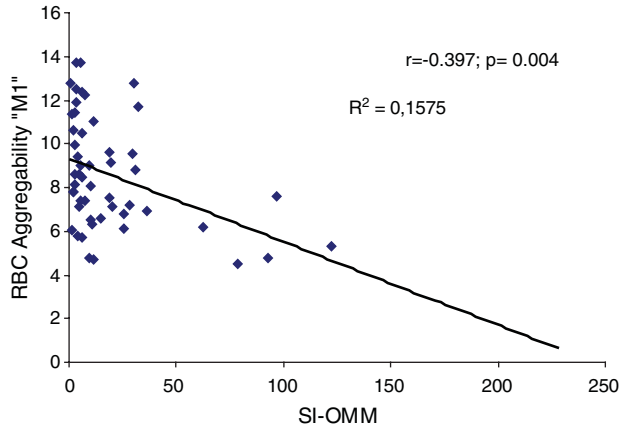

Fig. 2. Correlation between RBC aggregability index 'M1' and insulin sensitivity.

3. Results

Figures 1 and 2 show that the Myrenne indexes of red cell aggregation were negatively correlated to SI (M; $r = -0.456$; $p = 0.0007$; M1; $r = -0.397$; $p = 0.004$). Fasting insulin levels (Ib) were also correlated to M and M1, but that this correlation was weaker (M; $r = 0.2711$; $p = 0.05$; M1; $r = 0.373$ $p = 0.007$). Plasma viscosity was not correlated to SI ($r = 0.146$, NS).

A stepwise regression analysis selects M as the best correlate of SI (0.456; $p = 0.0007$) and M1 as the best correlate of Ib ($r = 0.373$ $p = 0.007$).

4. Discussion

This study confirms with another approach our previous finding that RBC aggregability is a marker of insulin resistance. There is also a correlation between insulinemia and RBC aggregation but it is

weaker. This suggests that aggregability is rather related to insulin resistance than the compensatory hyperinsulinemia that occurs when insulin sensitivity decreases. By contrast, no clear correlation with plasma viscosity is observed.

The OMM approach allows a precise and reliable measurement of SI validated against clamp [18], isotopic measurements [16], and minimal model analysis of the intravenous glucose tolerance test [1], even in diabetics [10].

It was thus attractive to try to confirm our previous reports of the hemorheological tableau of insulin resistance with this alternative approach. Interestingly, we largely confirm that a decrease in insulin sensitivity is associated with an impairment in viscosity parameters, but the factor that exhibits the strongest relation is erythrocyte aggregation. Consistent with our previous report with intravenous glucose tolerance test [12, 26], it seems that an increase in plasma viscosity rather reflects a true impairment of glucose tolerance, *i.e.* a further step in the insulin resistance syndrome. RBC aggregation seems to be an earlier, nonspecific but very sensitive, marker. The fact that, as reported by our group [28–30], fibrinogen is negatively correlated to insulin sensitivity may contribute to explain this finding.

In conclusion, this study with an alternative well validated measurement of insulin sensitivity leads to think that an increase in RBC aggregation is an early sign of insulin resistance, while other hemorheologic disturbances reflect a more marked impairment, ie presumably a more advanced stage of the disease.

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The authors comply with the Ethical Guidelines for Publication in Clinical Hemorheology and Microcirculation as published on the IOS Press website and in Volume 44, 2010, pp. 1–2 of this journal [4].

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