Multivariate analysis of relationships between insulin sensitivity and blood rheology: Is plasma viscosity a marker of insulin resistance?

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Abstract. We previously reported in populations exhibiting all the spectrum of insulin sensitivity (SI) values correlations between SI and blood viscosity η suggesting that high η is an additional symptom of the insulin resistance syndrome. However, due to the elevation of insulinemia (I) which is usually associated with insulin resistance it remained to determine whether this relationship was explained by SI or I. We analyzed SI with the minimal model procedure in 108 nondiabetic subjects and analyzed correlations of SI with blood rheology (η , RBC aggregation and rigidity). Across quartiles of SI (defined after log transformation since distribution of SI was not normal), hematocrit and red cell rigidity remained stable, while aggregability and plasma viscosity (η p) increased in the lowest quartile. SI was correlated to only two rheological parameters: ηp (r=-0.280, p=0.005) and Myreme index M1 (r=-0.219, p=0.044). Among SI, I, age and BMI multivariate analysis selected only BMI as a determinant of either whole blood viscosity (η wb: r=-0.301, p=0.004) and RBC disaggregation threshold (γ D: r=-0.331, p=0.013), only I as determinant of M1 (r=0.254, p=0.03), and a combination of BMI (p=0.009) and SI (p=0.007) for ηp . Although age and obesity are factors of hyperviscosity, the hemorheological disturbances found in insulin resistance are not fully statistically "explained" by those two factors. While hyperaggregability (measured with M1) is rather related to hyperinsulinism, ηp is influenced by SI and should be further investigated as a simple marker for the follow up of insulin-resistant states.

Keywords: Blood viscosity, plasma viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, insulin sensitivity, insulin resistance, minimal model

1. Introduction

The insulin resistance syndrome represents a widely accepted explanation of the classical association of lipid disorders, obesity, impaired glucose tolerance, hypertension and increased cardiovascular risk [1–9]. There are reports of correlations between insulin resistance and abnormalities of blood rheology [10–14] and high fibrinogen [15–17]. Actually it is clear that the theory of insulin resistance as the common explanation of all this picture is probably an oversimplification [9]. An alternative hypothesis assuming that endothelial dysfunction may be in fact the underlying mechanism explaining both

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insulin resistance and the cardiovascular disease [18] is supported by several lines of clinical and experimental evidence [19–21]. Notwithstanding this controversy, insulin resistance clearly appears to be a frequent clinical situation which is statistically strongly associated with cardiovascular morbidity and mortality [1–9].

Given the fact that blood rhoology emerges as an independent cardiovascular risk factor [22–27] this relationship between SI and rhoology may be more than a statistical curiosity and may have clinical implications, as suggested, for instance, by a recent work of Häieggen [14] which largely confirmes our earlier findings [10–12].

However, most of the evidence supporting the involvement of insulin resistance in cardiovascular risk consists of studies in which crude fasting insulin levels were used as markers of insulin resistance [28-33]. One could critically interpret these reports as rather demonstrating that hyperinsulinemia, whatever it means, is statistically associated with cardiovascular risk [33], regardless insulin sensitivity itself. In fact, consistent with these remarks, the validity of fasting insulin (and various related simplistic indices for detecting insulin resistance) has been recently questioned [34]. Insulin has been shown to mirror (in physiological conditions) insulin sensitivity, due to an homeostatic feedback loop that maintains constant the product insulinemia (I) × insulin sensitivity (SI) [35,36], so that insulin sensitivity, physiologically defined as the dosc-response relationship, and expressed in min⁻¹/ $(\mu U/ml) \times 10^{-4}$ can be grossly evaluated as the ratio SI = 40/I [37-39]. Actually indices based on fasting insulin have been demonstrated to correctly fit with SI measurements in some situations like polycystic ovary syndrome [40] or nondiabetic obesity [41], suggesting that they really could help to evaluate SI over a wide range of clinical situations [42]. However, there are clearly situations of complete discrepancy between SI and indices based on I, such as trained athletes [43], reactive hypoglycemia [44], and diabetes [44], so that the general use of I as a mirror of SI should not be recommended outside of conditions where its validity has been well demonstrated [44].

These considerations are important for our purpose of studying the relationships between SI and rheology. Clearly, whether the previously reported hemorheological disturbances of the insulin-resistance syndrome [10–17] are related to low SI or to high I is still unclear. Theoretically, both can be expected to affect blood rheology. Low SI induces a lot of metabolic disturbances [1,6] affecting carbohydrate, lipid and fibrinogen [15–17,45] metabolism, while insulin exhibits direct effects on the red cell rheology [46–48].

Therefore, we conducted this study in order to determine: (a) whether correlations between SI and blood viscosity are explained by SI or I; (b) which hemorheological parameter (if any) is directly related to SI after the statistical influence of I has been "neutralized" by multivariate analysis.

2. Methods

Subjects used in this study were 108 nondiabetic subjects (38 males, 69 females) whose clinical characteristics are shown in Table 1. They were selected in an outpatient unit of Endocrinology and metabolism where they had to perform a measurement of insulin sensitivity, either for detecting low values of SI or for detecting unusually high values of SI. They thus cover all the spectrum of SI values found in physiology and pathology.

 $\label{eq:Table I} \mbox{Table I}$ General characteristics of study subjects (mean \pm SEM)

Age	Weight	Height	ВМІ	WHR	Fat mass
(years)	(kg)	(m)	(kg/m^2)		(kg)
$\overline{35.75 \pm 1.25}$	81.35 ± 2.25	1.67 ± 0.01	29.08 ± 0.79	0.88 ± 0.028	34.74 ± 2.58

 \overline{BMI} ; body mass index (= weight/height²); WHR: waist to hip ratio,

2.1. Hemorheological measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at high shear rate (1000 s⁻¹) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [49]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [50]. The coefficient of variation of this method ranged between 0.6 and 0.8% [51]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [52]. Dintenfass' 'Tk' index of erythrocyte rigidity was calculated [53]. RBC aggregation was assessed with the Myrenne aggregometer [54] which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at 600 s⁻¹) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s⁻¹). The hematocrit/viscosity (h/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [55] and Stoltz [56], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above.

The SEFAM aggregometer was used for a more precise assessment of RBC aggregation. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [57,58]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time (TA) is the reciprocal of the initial slope (calculated between 0.5 and 2 s after the shear has stopped). The aggregation index at 10 s (S10) is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 seconds and the aggregation index at 60 s (S60) is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 60 seconds. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s⁻¹ to 7 s⁻¹. The total disaggregation threshold (γ S) is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate (γ D) is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate).

2.2. Frequently sampled intravenous glucose tolerance test (FSIVGTT)

A cannula was placed in the cephalic vein at the level of the cubital fossa for blood sampling at various times, while glucose was administered via the contralateral cephalic vein. Glucose (0.5 g.kg⁻¹, solution at 30%) was slowly injected over 3 min. Insulin (0.02 units/kg⁻¹ body weight, i.e., 1–2 units) was injected into the vein contralateral to the one used for sampling, immediately after 19 min. Blood samples were drawn twice before the glucose bolus and at 1, 3, 4, 6, 8, 10, 15, 19, 20, 22, 30, 41, 70,

90 and 180 min following glucose injection. Times 1 and 3 min were used for the determination of the insulin early secretory phase [59]. The other times were necessary for minimal model calculations.

2.3. Glucose disposal coefficient (Kg)

The least square slope of the log of the absolute glucose concentration, between 4 and 19 minutes after the glucose bolus, was used as an index of glucose tolerance, Kg4–19. This Kg value describes glucose disposal by tissue and depends on three factors: insulin release, insulin sensitivity, and glucose effectiveness independent of insulin.

2.4. Measurement of insulin sensitivity and glucose effectiveness

Minimal model analysis of FSIVGTT was according to Bergman [13] with the home-made software "TISPAG", which uses a nonlinear least square estimation, from the Department of Physiology, University of Montpellier I [60–62]. This program gave the values of insulin sensitivity (SI) and glucose effectiveness (Sg). SI is a measurement of the influence of plasma insulin to change glucose's own effect on glucose concentration. Sg is the fractional disappearance rate of glucose, independent of any insulin response. This parameter Sg was actually broken down into its two components [63]: the contribution of hyperglycemia *per se* to tissue glucose utilization and the effect of basal insulin on glucose uptake. The basal insulin component of Sg is termed the basal insulin effect (BIE) and can be calculated as the product of basal insulin, Ib, and SI. Thus the contribution of non-insulin-dependent glucose uptake (glucose effectiveness at zero insulin, GEZI) to glucose uptake is the difference between total Sg and BIE. $GEZI = Sg - (Ib \times SI)$.

2.5. Laboratory measurements

Samples were analyzed for plasma insulin by radioimmunoassay (kit SB-INS1-5 from the international CIS). The within-assay coefficient of variation (CV) for insulin was determined by repetitive measurements of the same sample and was 6.6%; the between-assay CV was 6.2%. The sensitivity (lowest detectable value) was $<1~\mu\text{U/ml}$. Plasma glucose was measured with a Beckman glucose analyzer, with coefficients of variation of 8.3% (within-assay) and 7.9% (between-assay). Fibrinogen was measured with the Clauss method.

2.6. Statistics

Data are expressed as means \pm SE. To detect differences between parameters represented by a single measurement, non-parametric tests for unpaired (Mann-Whitney) and paired (Wilcoxon) data were used as appropriate. Correlations were performed by Pearson analysis and multiple regression analysis. Sensitivity was calculated as the number of truly positive subjects divided by the sum of true positive and false negative, that sum representing the total number of insulin resistant patients in the sample of subjects. The specificity was calculated as the number of truly negative subjects divided by the sum of false positive and true negative. The positive predictive value was calculated as the number of truly positive subjects divided by the sum of true positive and false positive. The negative predictive value was calculated as the number of truly negative subjects divided by the sum of true negative and false negative ones. All these four indexes were expressed as percentages. Normality of parameters was assessed with the normality test of Kolmogorov and Smirnov. This test gives a K-S distance and a p value that allow

Table 2
Laboratory measurements in the 108 subjects of the study

Baseline insulin (µU/ml)	11.3 ± 0.9
Peak insulin I_{1+3} (μ U/ml)	102.3 ± 7.7
$\text{Kg (min}^{-1} \times 10^{-2})$	2 ± 0.1
Sg (min $^{-1} \times 10^{-2}$)	2.69 ± 0.11
$SI (min^{-1}/(\mu U/mI) \times 10^{-4})$	7.2 ± 1
Cholesterol (mmol/l)	4.2 ± 0.4
Triglycerides (mmol/l)	1.51 ± 0.29
HDL Chol (mmol/l)	1.07 ± 0.15
Fibrinogen (g/l)	3.26 ± 0.13

Kg: glucose tolerance, Sg: glucose effectiveness. Sl: insulin sensitivity.

to conclude that the test "passes" or "fails". A test that fails indicates that the data varies significantly from the pattern expected if the data was drawn from a population with a normal distribution. A test that passes indicates that the data matches the pattern expected if the data was drawn from a population with a normal distribution. P < 0.05 was considered significant.

3. Results

Values of SI covered all the range of insulin sensitivity, between 0.001 and 43 min⁻¹/(μ U/ml) × 10⁻⁴. As expected [13] SI did not exhibit a normal distribution with the Kolmogorov–Smirnov test (K–S distance = 0.2405; $P \le 0.0001$; failed), neither did basal insulinemia (K–S distance = 0.2200; $P \le 0.0001$; failed) and glucose effectiveness (K–S distance = 0.1098; P = 0.0027; failed). Similarly, hemorheological parameters exhibited a nonnormal distribution: blood viscosity (K–S distance = 0.1188; P = 0.0013; failed); plasma viscosity (K–S distance = 0.0896; P = 0.0485; failed); 'Tk' (K–S distance = 0.1087; P = 0.0058; failed); hematocrit (K–S distance = 0.1245; P = 0.0007; failed), unless they were log-transformed. Only 'M' (K–S distance = 0.0601; P = 0.6017; passed) and 'M1' were normally distributed.

The nonnormal distribution pattern of SI prompted us to use log transformation prior to defining quartiles of distribution. With this procedure the limit of the upper quartile was $9.5 \, \mathrm{min^{-1}/(\mu U/ml)} \times 10^{-4}$ and the limit of the lower quartile was $1.1 \, \mathrm{min^{-1}/(\mu U/ml)} \times 10^{-4}$. Comparison of hemorheological parameters across these quartiles of distribution of SI is shown in Tables 4, 5, and 6. Hematocrit and red cell rigidity remained the same whatever the quartile, while aggregability M (p < 0.02) and M1 (p < 0.01) as well as plasma viscosity (p < 0.001) increased in the lowest quartile (Fig. 1). Similarly, both total and partial disaggregation thresholds were significantly higher in the lowest quartile of SI. SI was correlated to only two rheological parameters: ηp (r = -0.280, p = 0.005) and Myrenne index M1 (r = -0.219, p = 0.044). Among SI, I, age and BMI multivariate analysis selected only BMI as a determinant of either whole blood viscosity (η wb: r = -0.301, p = 0.004) and RBC disaggregation threshold (γ D: r = -0.331, p = 0.013), only I as determinant of M1 (r = 0.254, p = 0.03), and a combination of BMI (p = 0.009) and SI (p = 0.007) for ηp .

Since ηp appears in these results to be the hemorheological parameter the most closely related to SI, we defined also quartiles of distribution of ηp after log transformation. The boundary of the upper quartile of ηp was 1.45 mPa.s. SI was lower (3.48 \pm 0.97 min⁻¹/(μ U/ml) \times 10⁻⁴) in the 28 subjects whose ηp was >1.45 mPa.s than in the 80 others (8.6 \pm 1.37 min⁻¹/(μ U/ml) \times 10⁻⁴) p < 0.04. In the lowest quartile

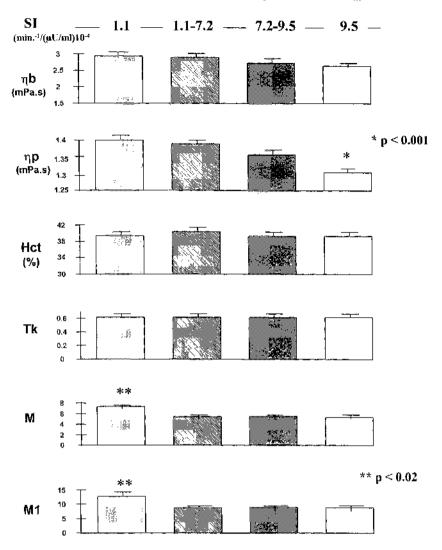


Fig. 1, Mean values (± SEM) of hemorheological parameters in the 4 quartiles of distribution of insulin sensitivity (SI).

of ηp there were 10 low values of SI (<1.1 min⁻¹/(μ U/ml) × 10⁻⁴), i.e., 35% vs 10 insulin resistant subjects in the 3 other quartiles put together (i.e., 12.5%), indicating that there is a 2.8-fold increase in the incidence of insulin resistance in subjects with high ηp above 1.45 mPa.s (p=0.014, Fisher's exact test). Table 7 shows that patients with high ηp have a higher body mass index and a higher insulinemia either at baseline or after iv glucose. Interestingly, their glucose effectiveness was not different from patients of the other quartiles. While patients with high ηp also exhibit higher whole blood viscosity, their red cell deformability and aggregation parameters were similar (data not shown).

These results can also be expressed in terms of sensitivity and specificity of ηp for predicting insulin resistance. Among 22 'insulin-resistant' subjects there were 9 patients with $\eta p > 1.45$ mPa.s and 13 with $\eta p <$ this value. Among the 86 others with 'normal' SI there were only 10 with $\eta p > 1.45$ mPa.s and 76 with $\eta p < 1.45$. Thus, the sensitivity of ηp for detecting insulin resistance is 9/22 = 0.409, the specificity 76/86 = 0.884, the positive predictive value 9/(9+10) = 0.474 and the negative predictive value 76/(13+76) = 0.854.

Table 3
Hemorheologic parameters in the 108 subjects of the study

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Blood viscosity ηb (mPa,s)	2.79 ± 0.04
Plasma viscosity ηp (mPa.s)	1.37 ± 0.01
Venous hematocrit (%)	39.6 ± 0.48
Erythrocyte rigidity 'Tk'	0.62 ± 0.01
Erythrocyte aggregation 'M'	5.77 .1 0.24
Erythrocyte aggregation 'M1'	9.6 ± 0.37
Aggregation kinetics 'Tf'	30.84 ± 1.58
Aggregation kinetics "TA"	1.89 ± 0.13
Aggregation kinetics 'S10'	31.5 ± 1.43
Aggregation kinetics 'S60'	48.7 ± 1.23
Disaggregation $\gamma S(s^{-1})$	139.3 ± 12.5
Disaggregation γD (s ⁻¹)	69.5 ± 3.79
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Table 4 Comparison of general characteristics of study subjects (mean \pm SEM) classified in 4 quartiles of insulin sensitivity

Quartile	Age	Weight	Height	BMI	WHR	Fat mass
of SI	(years)	(kg)	(m)	(kg/m^2)	(kg)	(%)
$\overline{\text{Lowest} < 1.1 \ (n = 22)}$	35.32 ± 2.88	85.39 ± 5.29	1.63 ± 0.02	31.69 ± 1.72	0.97 ± 0.051	30.22 ± 4.1
1.1-7.2 (n = 22)	36.86 ± 2.79	96.66 ± 4.9	1.6 ± 0.02	34.08 ± 1.8	0.96 ± 0.08	44.01 ± 4
7.2-9.5 (n = 38)	35.68 ± 2.29	78.52 ± 2.75	1.68 ± 0.02	27.7 ± 0.93	0.83 ± 0.03	36.01 ± 4.28
Highest > 9.5 ($n = 26$)	35.28 ± 2.27	69.5 ± 4.08	1.68 ± 0.019	24.7 ± 1.4	0.75 ± 0.025	20.07 ± 7.57

BMI: body mass index (-- weight/height²); WHR: waist to hip ratio.

Table 5

Comparison of metabolic parameters and glucose disposal parameters of study subjects (mean + SEM) classified in 4 quartiles of insulin sensitivity

Quartile of SI	Ib	I_{1+3}	Kg	Sg	SI	Chol	TG	Fg
Lowest < 1.1	16.09	134.3	1.54	2.09	0.43	6.47	2.3	3.4
(n = 22)	±2.48	± 22.04	± 0.14	± 0.16	± 0.07	± 0.71	± 0.9	± 0.3
1.1-7.2	11.62	119.48	1.66	2.39	1.95	5.62	1	3.2
(n = 22)	± 1.3	± 20.95	± 0.15	± 0.227	± 0.09	± 0.34	± 0.17	± 0.2
7.2-9.5	10.6	91.3	2,2	3	5.57	5.94	1.27	3.3
(n = 38)	±1.7	± 10.02	± 0.23	:-0.21	± 0.31	± 0.42	±0.2	±0.2
Highest >9.5	7.96	74.5	2.36	3.00	19.9	5.71	1,26	3.04
(n = 26)	±0.70	⊢8.7	±.0.20	± 0.26	±2.8	± 0.54	± 0.4	.10.2

Ib: baseline insulin; T_{1+3} : insulin peak (sum of postchallenge values at 1 and 3 min); Kg: glucose tolerance, Sg: glucose effectiveness; SI: insulin sensitivity; Chol: cholesterol (mmol/l); TG: triglycerides (mmol/l); Fg: fibrinogen (g/l).

4. Discussion

Our results show that insulin sensitivity is statistically associated with two hemorheological parameters: plasma viscosity and red cell aggregability. While aggregation appears in multivariate analysis to be rather related to hyperinsulinemia than to low insulin sensitivity, plasma viscosity is independently correlated to insulin sensitivity.

Table 6
Comparison of hemorheological parameters of study subjects (mean \pm SEM) classified in 4 quartiles of insulin sensitivity

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Quartile	ηb	ηр	Het	Tk	М	M1
of SI						
Lowest $< 1.1 (n = 22)$	2.94 ± 0.15	1.40 ± 0.02	39.49 ± 1.50	0.62 ± 0.02	7.40** ±: 0.71	$12.9^{***} \pm 1.13$
1.1-7.2 (n = 22)	2.90 ± 0.10	1.39 ± 0.03	40.48 ± 0.66	0.62 ± 0.02	5.46 ± 0.50	8.85 ± 0.75
7.2-9.5 (n = 38)	2.73 ± 0.05	1.36 ± 0.02	39.36 ± 0.89	0.62 ± 0.03	5.52 ± 0.31	9.04 ± 0.48
Highest > 9.5 ($n = 26$)	2.65 ± 0.07	$1.31^{***} \pm 0.02$	39.29 ± 0.69	0.62 ± 0.01	5.31 ± 0.5	8.93 ± 0.61
	NS	***p < 0.001	NS	NS	p < 0.02	p < 0.01
Quartile	TA	Tf	S10	S60	γ S	γD
of St	(s)	(s)			(s^{-1})	(s^{-1})
Lowest $< 1.1 (n = 22)$	1.42 ± 0.15	23.8 ± 2.08	34.45 ± 1.67	50.3 ± 1.15	219.7* ± 48.67	89.4** ± 9.65
1.1-7.2 (n = 22)	1.9 ± 0.27	27.58 ± 2.45	30.2 ± 2.04	46.7 ± 3.02	122.03 ± 9.5	66.42 ± 5.12
7.2-9.5 (n = 38)	2.04 ± 0.26	35.6 ± 3.3	32.2 ± 3.60	49.6 ± 2.78	124.38 ± 15.16	67.12 ± 7.58
Highest > $9.5 (n = 26)$	2.09 ± 0.30	33.05 ± 2.38	28.8 ± 1.33	47.69 ± 1.18	106.6 ± 7.76	57.9 ± 2.48
					*p < 0.05	**p < 0.01

 η b: blood viscosity; η p: plasma viscosity; Het: venous hematocrit; 'Tk': erythrocyte rigidity index; 'M' and 'M1' erythrocyte aggregation indices calculated respectively at stasis and low shear rate with the Myrenne; 'Tf', 'TA', 'S10', and 'S60': parameters quantifying erythrocyte aggregability with the SEFAM AFFIBIO erythroaggregometer; γS: total disaggregation threshold; γD: partial disaggregation threshold, ', ** and ***: comparison vs closest quartile.

Table 7
Comparison of anthropometric and metabolic characteristics of study subjects (mean \pm SEM) classified in 4 quartiles of plasma viscosity: highest quartile vs the 3 others put together

Quartile	Age	Weight	Height	BMI	WHR	Fat mass	lb.
of ηp	(years)	(kg)	(m)	(kg/m^2)	(kg)	(%)	
3 lower < 1.45	34.27	79.75	1.68	28.19	0.88	35.9	9.56
(n = 80)	± 1.51	± 5.29	± 0.012	± 0.99	± 0.056	±4.23	± 0.47
Highest > 1.45	40.87	90.76	1.67	32.7	0.90	32.83	13.23
(n = 28)	±2.55*	±4.5*	± 0.02	±1.56**	±0.03	±4.07	±2.13**
Quartile	I_{1+3}	Kg	Sg	SI	Chol	TG	Fg
of ηp	· · · · · · · · · · · · · · · · · · ·						
3 lower < 1.45	89.9	1.96	2.67	8.23	4.82	1.21	3.16
(n = 80)	± 6.8	± 0.14	± 0.15	± 1.41	± 0.41	± 0.9	± 0.11
Highest >1.45	129.7	1.84	2.33	4.12	4.85	2.69	3.47
(n = 28)	$\pm 25.23*$	± 0.14	± 0.18	$\pm 1.11^{*}$	± 0.61	± 1.48	± 0.3

BMI: body mass index (= weight/height²); WHR: waist to hip ratio; Ib: baseline insulin; I_{1+3} : insulin peak (sum of postchallenge values at 1 and 3 min); Kg: glucose tolerance; Sg: glucose effectiveness, SI: insulin sensitivity; Chol: cholesterol (mmol/l); TG: triglycerides (mmol/l); Fg: fibrinogen (g/l). Comparison: p < 0.05; **p < 0.02.

To some extent, plasma viscosity thus appears to be a marker of insulin resistance in such a sample of patients. When it is found to be above 1.45 mPa.s, there is a 2.8-fold increase in the incidence of insulin resistance which is then found in 35% of subjects, while it is found in only 12.5% of subjects with normal or high values of insulin sensitivity. While the sensitivity of plasma viscosity for detecting insulin resistance is poor (40.9%), as reflected also by a low positive predictive value (47.4%), there is a quite high specificity (88.4%) and negative predictive value (85.4%), at least in subjects similar to this sample of unselected outpatients attending to an endocrinology unit. In other terms, plasma viscosity

considered alone is surely not an interesting tool for detecting insulin resistance, but when it is found to be higher than 1.45 mPa.s it is highly suggestive for this syndrome. This simple and cheap marker is not sensitive but exhibits a rather good specificity in the population studied here.

Obviously, the first point that should be discussed is the relevance of our sample of 108 subjects, which is not representative of the general population but rather reflects the average population attending to an endocrinology hospital outpatient unit, i.e., people in whom some degree of endocrine and metabolic disturbance could be expected. In fact, all the lower quartile of this population is clearly insulin resistant, since the upper boundary of this quartile (which is found at $1.1 \, \text{min}^{-1}/(\mu \text{U/ml}) \times 10^{-4}$) is similar to the lowest limit (mean $-2 \, \text{SD}$) of a control group we previously defined in a physiological study [62,64,65]. By contrast, the upper quartile, which exhibits no hemorheological specific pattern, represents cases of very high values of insulin sensitivity as can be found in reactive hypoglycemia [64], athletes [65] or moderate lower body overweight [66]. Thus, this sample gives a picture of all the spectrum of insulin sensitivity, with an increased representation of extreme values in the lower and the upper range, which respectively represent the lower and the upper quartile. We think that such a sample is suitable for investigating the effects of abnormal insulin sensitivity values on blood rheology, since there is a strong representation of these values.

In a preceding work we aimed at selecting another sample designed to be representative of the average population [10–12]. We found that insulin sensitivity was negatively correlated to whole blood viscosity. This finding has been confirmed by others [14]. We concluded that whole blood viscosity was likely to mirror a host of various metabolic parameters controlled by insulin sensitivity (e.g., circulating lipids, glycemia, water and ion status, blood pressure, etc.), and its determinants (mostly body composition). A further study by Häieggen and coworkers [14] is in agreement with this assumption, since these authors, who observe negative correlations between glucose disposal rate and whole-blood viscosity at low and high shear rates, also report that blood viscosity correlates to scrum triglyceride and total cholesterol. Clearly these correlations may explain to some extent the relationship between glucose disposal and rheology [67–70].

In the sample studied here, the correlation between SI and whole blood viscosity already reported by us [10–12] and others [13,14] does not reach significance (p < 0.1). We think that the large representation in the upper quintile of subjects with high SI largely explains this negative result which contrasts with preceding reports.

By contrast, a parameter that appears to be interestingly related to insulin-resistant states is plasma viscosity. While all the other determinants of blood viscosity are no longer correlated to SI after multivariate analysis, plasma viscosity is the only factor of viscosity that exhibits a statistically independent relation to SI.

Obviously, the statistical relationship between SI and plasma viscosity is not very close, as shown by the low sensitivity (40.9%) and the low positive predictive value (47.4%) of ηp for detecting low SI. By contrast, this parameter exhibits a fair specificity (88.4%) and negative predictive value (85.4%). This data should be considered in the light of the controversy about simple markers of insulin sensitivity [34–44]. Clearly, there is a need of simple and unexpensive markers of insulin resistance for clinical follow-up of those states, since the popular indices derived from baseline insulin are not valid in situations where the feedback loop between insulin secretion and insulin sensitivity is disrupted, e.g., diabetes and some borderline disturbances of carbohydrate homeostasis [44]. In fact, there is no measurement of SI that is both simple and safe [34]. Only sophisticated and expensive techniques such as the glucose clamp or the minimal model are fully reliable [34]. Thus, plasma viscosity, which is likely to offer an integrated reflect

of the complex picture of metabolic disturbances associated to low SI, may be helpful in this context, in association with other clinical and routine laboratory measurements.

Consistent with this last assumption, we report in a separate work that exercise training of insulin resistant patients has marked effects on plasma viscosity parallel with an improvement in body composition (loss of fat mass) and a shift towards a higher ability to oxidize lipids at exercise [71]. Thus, plasma viscosity is both a rather specific (albeit poorly sensitive) marker of insulin resistance and a parameter that is improved by therapeutics aiming at increasing insulin sensitivity, such as training [71], slimming [72], lipid lowering [73–75]. Interestingly, it emerges nowadays as a true cardiovascular risk factor [23,25, 76]. Its usefulness in the follow-up of the insulin resistance syndrome in various situations remains to be studied.

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