

The hemorheological aspects of the metabolic syndrome are a combination of separate effects of insulin resistance, hyperinsulinemia and adiposity

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1. Abstract

The metabolic syndrome which is at high risk for diabetes and atherothrombosis is associated with hemorheologic abnormalities. Initially, insulin resistance was considered as the core of the syndrome. However, it becomes clear that the syndrome is a cluster in which the combined effects of obesity, insulin resistance, and hyperinsulinemia can be inconstantly associated, contributing to a various extent to a global impairment of blood rheology. We previously reported in 157 nondiabetic subjects that both obesity and insulin resistance increase red cell rigidity (Dintenfass's T_k) and plasma viscosity (η_p), and that whole blood viscosity at high shear rate ($\eta_b 1000 \text{ s}^{-1}$) reflects rather obesity than insulin resistance. In this study we aimed at defining the specific hemorheologic profile of insulin resistance and hyperinsulinemia by separating a sample of 81 subjects into 4 subgroups according to quartiles of insulin sensitivity (SI) (measured with the minimal model of an intravenous glucose tolerance test) and baseline insulin. Results show that 1) values of SI within the upper quartile are associated with low η_b due to low η_p ; 2) low SI regardless insulinemia is

associated with increased aggregation indexes ; 3) when low SI is associated with hyperinsulinemia (insulin the upper quartile and SI in the lower) there is a further increase in η_b due to an increase in η_p ; 4) neither SI nor insulinemia modify Hct. Thus hyperinsulinemia and insulin resistance induce hyperviscosity syndromes which are somewhat different, although they are associated most of the time. Low SI increases RBC aggregation while hyperinsulinemia increases η_p .

Key-words: Insulin resistance, insulin sensitivity, minimal model, metabolic syndrome, hemorheology, plasma viscosity, erythrocyte aggregability

2. Introduction

The metabolic syndrome (which includes lipid disorders, obesity, impaired glucose tolerance, hypertension and increased cardiovascular risk), is associated with abnormalities of blood rheology [1-2] and high fibrinogen [3]. Insulin resistance and a compensatory hyperinsulinemia have been suggested to be the “core “ of this syndrome [4].

Recently, Ferrannini [5] reported that insulin resistance and hyperinsulinemia, although they are most of the time found together, are sometimes dissociated and result in a slightly different syndrome. Thus, whether the previously reported hemorheological disturbances of the insulin-resistance syndrome are related to low insulin sensitivity (SI) or to high insulin (I) is still unclear. Theoretically, both can be expected to affect blood rheology. Low SI induces a lot of metabolic disturbances [4] affecting carbohydrate, lipid and fibrinogen metabolism, while insulin exhibits direct effects on the red cell rheology [6].

We previously reported in 157 nondiabetic subjects that both obesity and insulin resistance increase red cell rigidity (Dintenfass's T_k) and plasma viscosity (η_p), and that whole blood viscosity at high shear rate ($\eta_b 1000 \text{ s}^{-1}$) reflects rather obesity than insulin resistance [7]. In this study we aimed at defining the specific hemorheologic profile of insulin resistance and hyperinsulinemia by separating a sample of 81 subjects divided into 4 subgroups according to quartiles of insulin sensitivity (SI) (measured with the minimal model of an intravenous glucose tolerance test) and baseline insulin.

3 Material and methods

Subjects used in this study were 81 subjects aged from 19 to 62 years, divided into 4 subgroups according to quartiles of insulin sensitivity (SI) (measured with the minimal model of an intravenous glucose tolerance test) and baseline insulin whose clinical characteristics are shown on 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.

Patients were classified in three subgroups on the basis of measurements of insulin sensitivity and baseline plasma insulin as indicated below.

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 ($0.02 \text{ units/kg}^{-1}$ 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. Minimal model analysis of FSIVGTT was according to Bergman [8] with the home-made software "TISPAG ", which uses a nonlinear least square estimation, from the Department of Physiology, University of Montpellier I [9]. 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 . Based on the techniques used in our unit, as previously reported [7], quartiles of SI defined after log transformation due to their nonnormal distribution are displayed as follows. The upper limit of the lower quartile of SI was $1.1 \text{ min}^{-1}/(\mu\text{U/ml}) \times 10^{-4}$. The lower limit of the upper quartile of SI was $9.8 \text{ min}^{-1}/(\mu\text{U/ml}) \times 10^{-4}$. The lower limit of the upper quartile of insulinemia was $18 \text{ b } \mu\text{U/ml}$.

Laboratory measurements

Samples were analyzed for plasma insulin by radioimmunoassay (kit SB-INSI-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 $<1 \text{ } \mu\text{U/ml}$. 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.

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) [10]. The coefficient of variation of this method ranged between 0.6 and 0.8% [10]. RBC aggregation was assessed with the Myrenne aggregometer [11] 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}).

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. 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 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.

4. Results

Table 2 shows the comparison of anthropometric and body composition data among the 4 subgroups. While patients are matched for age, there is a tendency to increased visceral

adiposity (higher % of fat, higher BMI, higher WHR) when SI decreases and even more when Ib increases.

Results of the comparison of hemorheologic parameters show:

- 1) a continuous trend to increase for η_b which is low in the upper quartile of SI and increases progressively across quartiles of decreasing SI, and even more when low SI is associated to high Ib. The clearest explanation for this increase in η_b is a similar trend in η_p ; η_p is low in situations of elevated SI and high when Ib increases. Isolated insulin resistance with normal Ib does not modify it.
- 2) low SI regardless insulinemia is associated with increased “M1” aggregation index (“M” follows a similar trend with less marked differences).
- 3) Concerning red cell deformability, the rigidity index “Tk” does not exhibit significant differences but there are nonsignificant tendencies that would perhaps become significant on a larger sample. Hyperinsulinemia does not increase Tk and rather decreases it.
- 4) Neither SI nor insulinemia modify hematocrit which is exactly the same in the 4 subgroups.

5. Discussion

The results of this study show that there is an overall tendency for blood viscosity to increase across quartiles of insulin resistance, so that it decreases when SI decreases and even more if insulin is high. This is unrelated to hematocrit which is remarkably similar across quartiles. Red cell rigidity "Tk" exhibits a tendency to

increase in the lower quartile, but this tendency does not reach significance. Most of this effect is thus explained by plasma viscosity. This parameter appears to be lower in the higher quartile of insulin sensitivity but is similar in the three other quartiles: actually it is only increased when low SI is associated with hyperinsulinemia. On the other hand, the lower quartile of insulin sensitivity is characterized by higher RBC aggregation regardless insulinemia.

These results further help to delineate the complex interrelationships among insulin sensitivity, metabolic disturbances, and blood rheology. It is clear that the previously reported correlations between insulin resistance and blood viscosity cannot be simply explained. Various protocols have been used in order to clarify this picture. A role of hyperinsulinemia [7], of lipid disorders [12], of high blood pressure [13], of excess adipose deposits that are assumed to release many rheo-active substances [14] has been reported. On the whole, insulin resistance itself seems to have little influence on blood rheology, although hyperviscosity (at least to some degree) is surely an almost constant feature of the metabolic syndrome [1, 2, 7].

This study shows that the hemorheologic signs of hyperinsulinemia are probably not exactly the same as those of insulin resistance. Although both disorders are generally associated (in 60% of the cases according to Ferrannini [6]) the distinction between hyperinsulinic insulin-resistant individuals and normoinsulinic ones clearly appears in our results. Both disorders are able to impair blood fluidity, but they seem to have quite different effects. Red cell aggregability "M1" is increased by 60% when insulin sensitivity is situated in the lower quartile. It does not seem to further increase if insulin is high. By contrast, plasma viscosity is markedly

increased (+13%) when insulin is high, while it appears to be lower when insulin sensitivity is elevated. Thus, our previous report of a specific relationship between insulin resistance and plasma viscosity in multivariate analysis seems to indicate that low plasma viscosity is a marker of high SI (and probably exercise training) while low SI by its own does not markedly modify this parameter. Results on red cell deformability as assessed by Dintenfass's index 'Tk' are not conclusive but may become significant on a larger sample. Clearly, hematocrit is influenced by neither SI nor hyperinsulinemia.

On the basis of the large multicentric database of the EGIR study, Ferrannini and Balkau [5] show that both hyperinsulinaemia and insulin resistance identify a similar average phenotype, consisting of slightly older age, moderate obesity, increased percent fat mass and central fat deposition, higher systolic and diastolic blood pressure, dyslipidaemia (i.e. higher serum triglycerides, lower HDL-cholesterol but normal LDL-cholesterol levels), and lower rates of post-hepatic insulin clearance. Most of these abnormalities are significantly associated with the presence of hyperinsulinaemia or insulin resistance even after adjusting for sex, age and BMI. When hyperinsulinaemia is not associated to insulin resistance the endogenous glucose production is lower rather than higher in comparison with the rest of the population. The authors suggest that both insulin resistance per se and hyperinsulinaemia make an independent contribution to the metabolic syndrome. Insulin resistance, defined as resistance of glucose uptake to insulin action, is associated with resistance of endogenous glucose release and lipolysis to insulin action, while hyperinsulinaemia increases triglycerides (when it is associated to a low SI and thus an increased supply of FFA to the liver) and also clusters with raised blood pressure levels, due to the well known the excitatory effects of insulin per se on sympathetic nervous activity and the anti-natriuretic action of the hormone [15]. Fig. 1

summarizes this proposal of classification of the metabolic syndrome in three sub-syndromes, and represents an attempt to integrate in this scheme the hemorheologic disturbances reported here. The clustering of plasma viscosity and triglycerides together in the sub-syndrome “low SI plus hyperinsulinemia” may indicate that there is a causal link between these two abnormalities in this syndrome. However, this issue remains to be further studied. Plasma viscosity in most studies is closely dependent upon the lipoprotein profile [14].

Actually, Ferrannini's proposal to separate the three subsets of the metabolic syndrome (low SI + high Ib; low SI alone; high Ib alone) can be discussed if one keeps in mind R. Bergman's proposal to explain the natural history of the metabolic syndrome [15]. If this syndrome is due to the progressive worsening of a disorder initially located in the portal circulation and due to lipid-induced hepatic insulin resistance, these situations described on the basis of a large-scale cross-sectional study may rather reflect various stages of a similar disease than separate pathologic situations. After an initial hepatic disorder marked by high Ib despite normal whole body SI, a state of low SI and high Ib is initiated, followed by a decline in the insulin secretory capacity so that SI remains low and Ib is no longer elevated. Caution is thus required to interpret the profiles of these three sub-syndromes that may be in fact three stages of a similar disease.

Fat mass, plasma lipid profile, inflammatory status, are all likely to exert separate effects on blood rheology [14]. Direct effects of insulin on red cell rheologic properties have been reported [6]. Since all these abnormalities may markedly vary during the natural history of the metabolic syndrome, caution is required before proposing a simplistic picture of all these interrelationships.

On the whole, this study suggests that hyperinsulinemia and insulin resistance induce hyperviscosity syndromes which are somewhat different, although they are associated most of the time. Subjects whose insulin sensitivity is situated within the lower quintile have a lower red cell aggregability "M1" regardless their insulin levels. Plasma viscosity is lowered in situations of high SI and increased when high insulin levels are associated to low SI.

6. References.

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Table 1

General characteristics of study subjects (mean \pm SEM). Abbreviations: BMI: body mass index (=weight/height²); WHR: waist to hip ratio.

AGE	WEIGHT	HEIGHT	BMI	WHR	FAT MASS
(years)	(kg)	(m)	(kg/m ²)		(kg)
35.75	81.35	1.67	29.08	0.88	34.74
± 1.25	± 2.25	± 0.01	± 0.79	± 0.028	± 2.58

Table 2.

Comparison of anthropometric and body composition data among the 4 subgroups.

	higher quartile of SI (n=21)	two middles quartiles of SI (n=39)	low SI and normal insulinemia (n=13)	low SI and hyperinsulinemia (n=7)
Age	35.8±2.6	37.3±1.9	36.3±3.8	30.3±4.4
Weight	70.4±4.7	83.1±3.1	79.7±5.9	99.2±9.8
Height	1.68±0.02	1.68±0.01	1.65±0.03	1.62±0.05
BMI	25.1±1.7	29.3±1	29.2±1.7	37.2±2.4
% of fat	28.5±7	39.6±4	38.2±5	35.7±7
WHR	0.76±0.04	0.86±0.04	0.97±0.06	0.99±0.06

Table 3

Hemorheological parameters across quartiles of insulin sensitivity. The two middle quartiles of SI are put together and the lower quartile is divided in two subgroups: low SI and normal insulinemia and low SI and hyperinsulinemia. *** $p < 0.01$ vs higher quartile; ** $p < 0.03$ vs higher quartile; * $p < 0.001$ vs low SI and hyperinsulinemia;

	higher quartile of SI (n=21)	two middles quartiles of SI (n=39)	low SI and normal insulinemia (n=13)	low SI and hyperinsulinemia (n=7)
η_b [1000 s ⁻¹ mPa.s]	2.65±0.08	2.81±0.07	2.97±0.07***	3.06±0.07***
Hct (%)	39.2±0.7	39.3±0.6	39.9±1.6	39.2±3
η_p mPa.s	1.31±0.02	1.38±0.02**	1.37±0.02*	1.55±0.04***
Tk	0.62±0.02	0.62±0.01	0.65±0.02	0.57±0.04
M	5.3±0.5	5±0.3	7.7±0.9	6.9±1.3
M1	9±0.7	8.4±0.5	13.4±1.6***	12.8±1.6***

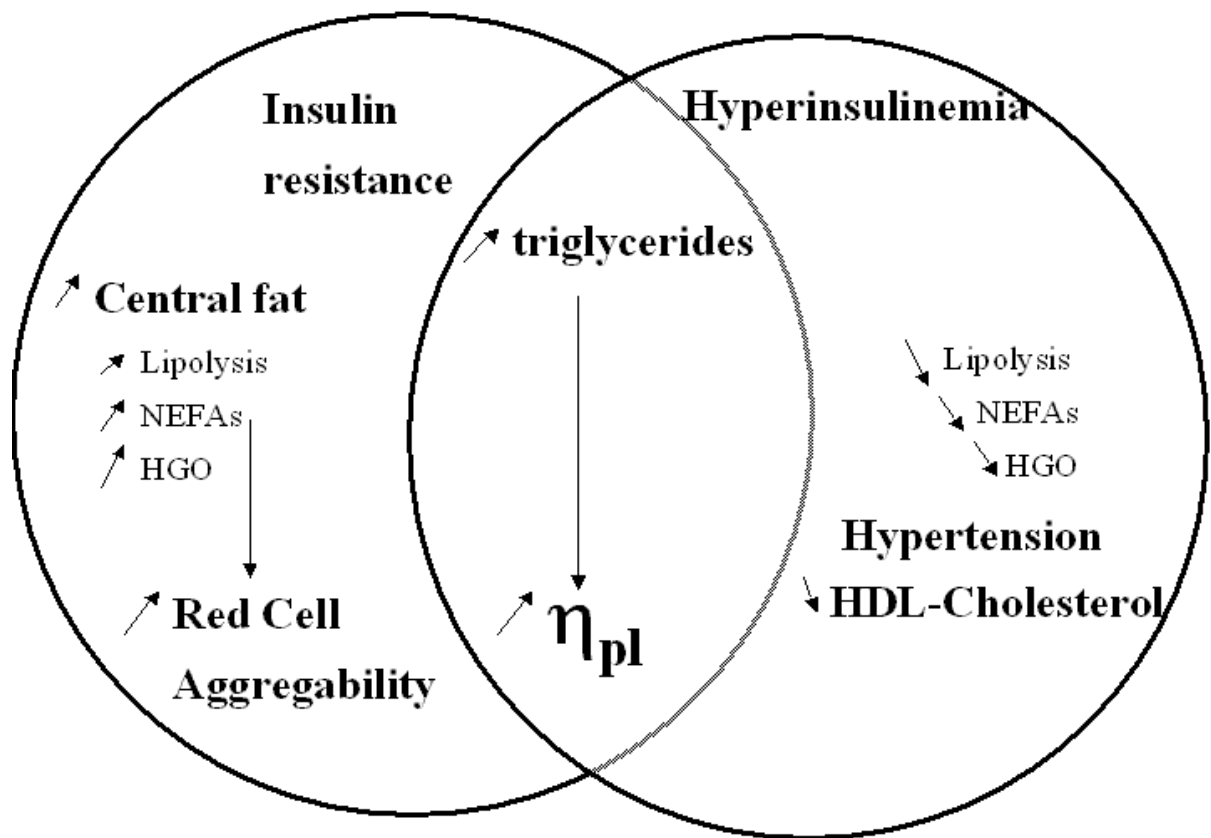


Fig.1

Schematic representation of the specific effects of insulin sensitivity and hyperinsulinemia on metabolism and blood rheology.