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Hormones, metabolism and body composition as major determinants of blood rheology: Potential pathophysiological meaning

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Abstract. The rheological properties of plasma and blood cells are markedly influenced by the surrounding milieu: physicochemical factors, metabolism and hormones. Acid/base status, osmolality, lipid status and plasma protein pattern are well known to exert a major influence. The oxidative stress induced by increased free radicals production decreases red cell deformability. Among circulating substances, the divalent cations magnesium and zinc improve red cell deformability probably via calcium antagonistic effects. Some metabolites like lactate or ketone bodies decrease red cell deformability, although the former has apparently the opposite effect in highly trained individuals. Endothelium-derived factors such as nitric oxide (NO) and several arachidonic acid derivatives modulate both RBC and white cell mechanics. Endothelium regulates also blood rheology via the release of PAI-1 which governs plasma fibrinogen levels. However, endothelium is not the only organ involved in the regulation of blood rheology: the kidney (by releasing erythropoietin which is a major "viscoregulatory" factor), the endocrine pancreas (via the action of insulin and glucagon on red cells), the adrenal gland (norepinephrine) and the endocrine heart (atrial natriuretic peptide) are also likely to exert important effects. Recently, increasing evidence is accumulating for a role of two other endocrine tissues in the regulation of blood rheology: the adipose tissue (free fatty acids, PAI-1, IL-6, leptin) and the pituitary gland (growth hormone–somatomedin axis, including the somatomedin carrier protein IGFBP1). These organs provide a link between body composition and hemorheology, since GH and somatomedins are major regulators of the body content in fat and water while the endocrine activity of fat mass is apparently proportional to its size. These mechanisms explain to some extent why many situations, either physiological (diet, exercise) or pathological (diabetes, uremia) are associated with marked changes in blood rheology that may in turn modify micro and macrocirculatory hemodynamics and the distribution of O_2 and fuels to tissues.

Keywords: Blood viscosity, plasma viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, hormones, insulin, insulin sensitivity, growth hormone, fibrinogen, metabolism

1. Introduction

There is increasing evidence that modifications of blood rheology are able to induce profound pathophysiological disturbances [1–3] that may explains why some large scale epidemiological studies that include viscosity and/or viscosity factors as potential explanatory variables evidence a strong statistical

link between rheology and both atherosclerosis and ischemia [4–7]. However, although a lot of information is available about most of these influences, we are not aware of a synthetic review summing up all the influences of physicochemical conditions, metabolism and hormones, on blood rheology. Therefore, we aimed in this review at providing a synthesis on this important physiological and pathophysiological issue.

A major biophysical background of blood rheology is the influence of physicochemical factors on viscosity. For instance, the influence of pH which actually appears to be different according to the age of cells, so that old erythrocytes may have a greater dynamic response to acid environment (e.g., spleen) which may cause them to be selectively trapped and eliminated from the circulation. Both pH and osmolality exert a biphasic influence described by a "u shaped curve" on red cell deformability the deformability being optimal in a physiological range and impaired beyond quite narrow physiological limits [8]. It is likely that most of this relationship is related to cell shape changes and surface/volume ratio alterations. The same effect seems to be observed with red cell aggregation, as shown by Tikhomirova [9] who studied aggregation after incubation at various pH levels from 7.2 to 7.8, and showed that alcalosis increases aggregability. A similar relationship is found with osmolality and aggregation [10].

On the whole, the best known humoral regulators of blood rheology are probably proteins [11,12] as recently reviewed by Donner [13]. Fibrinogen, as the major determinant of red cell aggregation, has been widely investigated [14–17]. This protective role of albumin is likely to result from its tertiary molecular structure, which can result in a too little size for inducing bridging among red cells, while when albumin has been heated it becomes polymeric and increases aggregation [13].

Lipoproteins exhibit strong correlations with rheologic factors, as evidenced on a population sample of 2211 subjects. Even after multivariate analysis, a rise of 100 mg/dl in serum cholesterol increases on the average plasma viscosity by 0.05 mPa.s [18]. In fact, 28% of the variance of of blood viscosity at low shear rate is likely to be explained by serum lipoprotein levels, with an opposite effect of low-density and high-density lipoprotein [19]. The issue of lipids can be even more complex, since essential polyunsaturated fatty acids of the omega 3 family (ω3PUFA) exert important physiological effects by improving RBC flexibility either in healthy volunteers [20,21] or in patients [22]. Vitamin E also given one month decreases RBC rigidity and improves microcirculation [23]. Finally, lipids and fibrinogen may act synergistically so that the effect of large triglyceride-rich lipoproteins is potentiated by fibrinogen [24].

The factor of viscosity which is the most clearly explained by these studies is plasma viscosity. Jung, after studying 2821 subjects included in the Aachen study, presented a formula predicting plasma viscosity from plasma proteins and lipids:

$$\begin{aligned}\text{plasma viscosity} = {} & 0.791 + 0.017 \cdot \alpha 2 \text{ macroglobulin} + 0.133 \cdot \text{fibrinogen} + 0.017 \cdot \text{IgM} \\ & + 0.11 \cdot \text{cholesterol}.\end{aligned}$$

In this analysis the strongest determinants are fibrinogen and cholesterol, while the effects of α2 macroglobulin and IgM are markedly lower [25]. More recently another similar formula was proposed by Etcrovic [26] on a smaller sample of 120 subjects including 30 controls. These authors give the following equation:

$$\begin{aligned}\text{plasma viscosity} = {} & 1.352 + 0.0167 \cdot \text{cholesterol (mmol)} + 0.0285 \cdot \text{fibrinogen (g/l)} \\ & + 0.0054 \cdot \text{triglycerides (mmol)} + 0.00318 \cdot \text{hematocrit} \\ & - 0.03 \cdot \text{HDL-cholesterol (mmol)}.\end{aligned}$$

Very interestingly, in this sample where all kinds of lipid disorders are well represented, the statistical weight of cholesterol is threefold higher than the weight of either fibrinogen and triglycerides.

Some studies have also be devoted to divalent cations. For example, magnesium has been shown to protect erythrocytes from *in vitro* experimental rigidification by several procedures [27,28]. Zinc, which *in vitro* increases the deformability of artificially hardened red cells [29], is frequenly low in the serum of sportsmen, this situation reflecting some degree of deficiency. Sportsmen with low serum zinc have a higher blood viscosity and an impairment in erythrocyte deformability [30] which is associated with a decrease in performance. Experimentally, a double blind randomized trial of oral zinc supply in healthy volunteers improves blood viscosity [31] while the effects on performance are not significant. Zinc seems also to reduce erythrocyte aggregation both *in vitro* and *in vivo* [32]. Iron is probably the most studied and the best known trace element. This interest is largely explained by the frequency of iron deficient states and by the possibility of treating them with iron supplementations. Almost 30 to 40% of the total body iron is stored under the form of ferritin and hemosiderin, while a lower amount is stored as transferrin [33]. Therefore, serum ferritin is a reliable marker of iron stores. Although a high ferritin value cannot rule out the existence of an iron-deficiency, a low ferritin value is highly specific of a deficiency. Experimental studies in iron-deficient rats have evidenced a lower erythrocyte flexibility that seemed to be related at least in part to a lower hemoglobin content of erythrocytes [34,35]. Athletes with low plasma ferritin exhibit a higher blood viscosity, a higher plasma viscosity, and a higher red cell aggregabilitity when compared to sportsmen with normal plasma ferritin. By contrast, we find no difference in either hematocrit or erythrocyte rigidity between these two subgroups [36]. It should be noted that Fe^{2+} deteriorates structure of RBC membranes [37] and thus induces more rouleaux networks.

Short-term hyperglycemia does not markedly impair blood rheology [38] unless extremely high concentrations (hundreds of mmol/l) which are never found in human disease are applied [39]. However, raised intracellular sorbitol resulting from chronic hyperglycemia may impair red cell deformability [40] but the experimental concentrations of sorbitol used in those studies are unlikely to be relevant to human disease.

However, diabetes is clearly a situation in which blood rheology is altered [41–44] although these alterations are quite moderate when the disease is well equilibrated [45,46].

Nevertheless, such situations of "covertly abnormal" blood rheology [47] are likely to induce some microcirculatory disturbances. Moreover, transient hyperglycemic spikes [48] raising up to 15.6 mmol induce alterations in red cell aggregation, plasma viscosity, fibrinogen and albumin which are associated with a measurable decrease in $TCPO_2$.

The issue of blood lactate is also rather complicated. While *in vitro* increased concentrations of this metabolite decreases red cell deformability [49], a rigidification of red cells during exercise is only found when blood lactate concentrations are higher than 4 mmol/l, i.e., the onset of acidosis [50]. In fact, in endurance trained athletes this effect is no longer found and, on the opposite, lactate may exert specific beneficial effects on red cell deformability [51,52].

High concentrations of ketone bodies are also likely to modify erythrocyte rheology, due to alteration in membrane properties, as evidenced by Peyreigne [53] in the case of a short-term ketogenetic diet.

2. Hormones and autacoids

Erythropoietin (EPO) is an endogenous hormone that regulates RBC production in the bone marrow. Recombinant human EPO is available and indicated for the treatment of anemia in a variety of medical

illnesses. In athletics, this agent is now used as an alternative to blood doping. Blood doping refers to the intravenous infusion of blood to increase oxygen-carrying capacity and thus to improve endurance performance. Typically, athletes draw and freeze the RBCs from 2 units of their own blood several weeks before a competition. This period allows the body to replace the RBC deficiency. The stored RBCs are then infused back into the body 1 week prior to competition. Controlled studies of this technique have suggested a 5% to 30% improvement in measures of endurance [54].

The use of EPO offers an alternative method to increase the concentration of circulating RBCs. EPO has advantages over blood doping in that it eliminates infusion risks (e.g., infection) and the need for refrigerated RBC storage. Limited experimental data suggest EPO efficacy to be similar to that obtained with blood doping [55]. Potential risks of EPO, as well as blood doping, are related to increased blood viscosity from an elevated hematocrit, i.e., hypertension, headache, and an increased risk for clotting, leading to coronary, pulmonary, or cerebral vascular occlusions [56,57].

One potential advantage of blood doping over EPO is that the "dose" of RBCs is known. The level of RBC production obtained with EPO is dose dependent but highly variable, and may continue for several days after the last administration. As a result, the hematocrit can be elevated to dangerous levels. It has been suggested that misuse of EPO may have led to a series of mysterious deaths in competitive cyclists between 1987 and 1990, in which 19 athletes died [58]. It seems clear that athletes are aware of the adverse effects of EPO related to clotting and are trying to stay ahead of the game. A raid of a cycling team at the 1998 Tour de France revealed not only EPO, but anticoagulant drugs as well [59].

Barbas [60] has investigated the effects of recombinant human erythropoietin (rhEPO) therapy on red cell filterability and membrane lipid composition in patients treated by haemodialysis for a long period (3, 9, and 18 months). At 9 months he observes a decrease in red cell filterability together with a rise in membrane cholesterol and total phospholipids. After 18 months, there is an increase in phosphatidylcholine/phosphatidylethanolamine ratio in the membrane. Besides, he observes no effect on red cell aggregation. A similar study has been conducted by Delamaire [61] in dialyzed chronic renal failure (CRF) patients after rhEPO versus renal transplantation. CRF patients have major disorders of blood rheology (red cells less deformable, increased plasma viscosity). Both rhEPO and kidney transplant correct these disturbances. In this case there are disorders of red cell aggregability which are not corrected by the treatment. A further by Boemi [62] evidences under a high dose (150 U/kg/wk) rhEPO treatment an increase in aggregability which is assumed to be potentially deleterious for patients.

2.1. Insulin

As soon as 1978, Juhan [63] reported that insulin improved hemorheologic abnormalities associated to diabetes. A direct effect of insulin on red cell fluidity was thus hypothesized. Obviously most of the hemorheologic improvement related to insulin treatment *in vivo* may be indirectly mediated by metabolic improvements. However, direct *in vitro* incubation of diabetic red cells by insulin repeatedly demonstrated a beneficial effect on deformability as measured by filtration yet this issue was challenged by some investigators when working on washed red cells [64,65]. It seems now well established that insulin really influences red cell rheology [66], via direct effects on the membrane [67] that include alterations of the lipid membrane bilayer composition and microviscosity, together with changes in membrane Na/K ATPase function [68].

According to the situation, the effects of insulin on red cell deformability may be different. For instance, very high supraphysiological levels *in vitro* decreased red cell deformability [66] as recently confirmed by Linde during insulin clamp experiments in which an increase in RBC rigidity in hypertensives was observed [69].

Glucagon, a major stress hormone which is involved in the recovery from hypoglycemia and exerts catabolic effects on body protein stores, has been shown to decrease red cell deformability [70].

Somatostatin is a tetradecapeptide that circulates in the blood [71]. It may induce strong circulatory changes as evidenced by a study showing an increase in peripheral blood flow in man [72]. In addition it interferes with platelet functions [73]. We reported a strong beneficial effect of this hormone on red cell deformability assessed by several techniques [74].

Among the recently postulated hormonal effects of the pancreatic C-peptide co-secreted with insulin, an increase in eNOS has been reported, resulting in diabetics in a fluidification of red cell membranes, an increase in renal function, blood flow redistributions and a reduction in Na/K ATPase pump function [75].

Catecholamines (norepinephrine and epinephrine) were first studied by Pfafferott who reported that *in vitro* norepinephrine and isoprenaline reduced erythrocyte deformability [76]. More recently, a more complicated picture emerged from the works of Hilario, Saldanha and Martins-Silva who demonstrated in human erythrocytes that although epinephrine is able to induce the formation of echinocytes, it also improves RBC deformability [77].

2.2. Leukotrienes and prostaglandins

Among arachidonic acid derivatives, some leukotrienes but not all [78,79] may impair red cell deformability. Prostaglandin E1 (and iloprost) improve red and white cell filterability *in vivo* [80,81]. PGE2 rigidifies red cells and increase their aggregability [81].

2.3. Nitric oxide (NO)

As recently reviewed [82,83] NO might have opposite effects on coagulation and rheology. Synergistically with prostacycline (PGI2), NO inhibits platelet aggregation and adhesion, reduces adhesion and migration of leukocytes, and exert marked vasodilatory effects in some territories. Atherosclerosis and hyperlipidemia decrease this endothelium-dependent vasodilation effect, via shifts in the expression of NO synthase (NOS) isoforms within the vessel wall. In fact, beside those well-established beneficial effects of vascular NO on circulation, there is on the opposite a strong deleterious effect of inflammatory cells – derived NO on the vessel wall, with possible effects on cell apoptosis and plaque fissuration [83].

Korbut [84,85] demonstrated that inhibiting NO production prevents the red cell rigidification resulting from experimental white cell activation. The team of Baskurt [86] studied experimental hypertension induced by the NO-synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME). This procedure both increases by 60% blood pressure and increases red cell rigidity and aggregation. If the NO-provider sodium nitroprusside SNP was added to those red cells it decreased on a dose-dependent manner RBC aggregation, with only a marginal effect on RBC deformability. Thus, NO exerts on hemorheology the same dual effects reported on circulation and atherogenesis [83]. Presumably leukocyte NO produced in inflammatory conditions (as studied by Korbut [84,85]) impairs red cell rheology while endothelial NO has the opposite effect. However, since blood rheology modifies endothelial shear stress which is a major regulator of NO secretion, caution is required in the interpretation of the relationships among NO and hemorheology: NO is likely to affect blood rheology and to be also regulated by it [82].

Purines may act via purinergic receptors on red cell rheology, as reported in the early eighties by Juhan et al. that ADP released by rigid red cells decreased the deformability of RBCs [63]. More recently it has been established that red blood cells can release ATP in response to hypoxia and hypercapnia. This ATP binds to purinergic P2 sites on endothelial cells leading to the release of NO and PGI2 [87].

Red blood cells can also release endothelin-1 (ET-1) together with endothelial and smooth muscle cells [87]. Despite its strong vasoregulatory effects, ET-1 seems, however, to have no measurable effect on blood viscosity and on red cell rheology [88].

Similarly, it is remarkable to notice that, despite the importance of intracellular calcium on red cell rheology, neither parathormone, calcitonin nor vitamin D_3 has any influence of blood rheology [89].

There is a large body of literature on the hemorheological effects of sex hormones. Most of this literature is related to oral contraceptives (OC), a hot topic because of its thrombogenic effects. Alterations of blood rheology in OC users have been first reported in 1971 by Aronson [90] and thus most precisely described in the early eighties by Lowe [91,92] and Buchan [93]. An elevation of fibrinogen levels [92–95] has been repeatedly observed and is likely to explain a rise in aggregability. However, it is interesting to compare the hemorheologic profile of the women of studies, taking contraceptives of the late nineties, with data reported two decades ago by the first authors who investigated this question. As reviewed in 1981 by Buchan [96], OC users in the late seventies were mostly characterized by a lower red cell deformability and a slightly higher whole blood viscosity despite normal values of plasma viscosity. Hematocrit was also reported to be increased in some studies [92] but not all [96]. The progestin component of the pill was assumed to be responsible for a rise in fibrinogen which explained most of this pattern. Actually, this finding on old progestin compounds of OC pills (mostly 19-nortestosterone derivatives), contrasts to some extent with the more recent physiological investigations [97] which evidenced in normally cycling women that estradiol levels were positively correlated to whole blood viscosity, plasma viscosity and fibrinogen, and negatively correlated to red cell deformability. In physiological conditions, therefore, estrogens were likely to impair blood fluidity. On the opposite, progesterone in physiological conditions had the opposite effect, decreasing both fibrinogen and blood viscosity, and increasing red cell deformability [97]. These physiological findings are in disagreement with a study performed ten years ago by Derham and Buchan, which demonstrated that pharmacologic intake of estrogens results in increased hematocrit and fibrinogen, while synthetic progestogens increase hematocrit and decrease red cell deformability [98]. Presumably, the modern evolution towards more physiological progestogens at lower doses tends to suppress these deleterious effects and to fit more closely with the physiological picture: this assumption is further supported by recent studies on blood rheology in OC-users [99,100]. These modern studies suggest that the recent, low dose, compounds are almost devoid of hemorheological side-effects [99,100], in contrast to older preparations with higher doses [96]. In a more recent study [101] we fail to observe any differences in blood viscosity and red cell deformability between OC-users and non users, suggesting that this evolution toward better-tolerated molecules and lower concentrations has been beneficial from an hemorheologist's viewpoint. However, there is still a moderately higher red cell aggregability. This finding suggests that the question of the hemorheologic effects of OC, although it has been investigated since 1971, still remains a potentially important field. Some recent findings on estrogen circulatory effects, such as their vasodilatory action which is mediated by NO synthesis and release by endothelial cells [102], may offer new areas of research for hemorheologists. Estrogen-response elements have been identified in the promoting region of the gene coding for the endothelial nitric oxide synthase [102]. Moreover, estrogens have been suggested to act more rapidly via membrane receptors, resulting in an increase in cytosolic Ca^{2+} in some cells [102]. Estrogens are also likely to exhibit antioxidant properties which may delay NO clearing from blood [102].

Another aspect of the relationships between sex hormones and rheology that has been extensively studied is menopause. However, the hemorheological profile of sex hormone deficiency at menopause is not clearly established. On the whole, there appears to be some degree of hemorheological disturbance, but

plasma viscosity seems to be more affected than red cell rheology [103,104]. Hormonal treatment by estrogens (either transcutaneous or oral) increases RBC rigidity by increasing membrane rigidity [105,106].

Actually, the purely physiological effects of the menstrual cycle are less known, despite some old studies showing changes in blood viscosity factors throughout the cycle [106,107]. Recent investigations with the cell transit analyzer evidence more deformable erythrocytes (shorter transit time) at ovulation and in luteal phase. These results contrast with previous works performed with the classical filtration methods, which actually are strongly influenced by the mean corpuscular volume of red cells [108].

3. Are they "hormonal axes" involved in the regulation of blood rheology

Literature summarized above indicates that a host of circulating factors (but not all) modify the rheologic properties of blood. In fact, many issues remain unclear at this time. However, given the number of informations collected, it could be speculated that all these interactions have a physiological relevance and can be classified as "regulatory axes". We make here-below an attempt to present all these axes.

3.1. The concept of viscoregulation

The oldest homeostatic concept in hemorheology is the concept of viscoregulation. Based upon his large clinical experience of hyperviscosity syndromes, Dintenfass [109] proposed that blood viscosity, just like all other biological parameters, is regulated by homeostatic loops. This author postulated at least two mechanisms by which any increase of a factor of blood viscosity would be sensed by a "viscoreceptor" which will in turn trigger a response resulting in a compensatory decrease in other factors, the most important in this regulation being probably hematocrit [109].

In diabetes, the importance of this mechanism has been underlined by two studies [130,131]. In this disease, situations leading to hyperviscosity (i.e., complications, even without nephropathy that can be a confusing factor if it leads to anemia) are associated with a decrease in hematocrit. In preeclampsia, the same has been observed [132].

More recently, one of these viscoregulatory mechanisms has been elucidated and carefully described by the team of Reinhart [133,134]. In a first clinical study, this team observed that increased plasma viscosity was associated with an inappropriate erythropoietin formation [133]. Later, the same team demonstrated that shear stress modulates erythropoietin secretion and explains unexpectedly low erythropoietin levels [134], and further analyzed on rats the mechanism of this regulation at the level of erythropoietin mRNA production. On the whole, these studies demonstrate that viscosity of plasma, as a factor of wall shear stress, is a major regulator of EPO production by the kidney, so that one of Dintenfass's "viscoreceptor"-mediated regulatory loops surely involves EPO. Accordingly, any increase in viscosity is likely to induce alterations in erythropoiesis leading to a reduction in hematocrit.

3.2. The endothelium-leukocyte-liver axis

The importance of this axis in hemorheology has been largely reviewed in recent papers [115,116]. Given its complexity, we shall not describe it in detail here. Both polymorphonuclear neutrophils and monocytes are able to release a host of biologically active substances that may interfere at various levels in the regulation of blood viscosity. Via cytokines like IL-6 they may induce a rise in fibrinogen which increases plasma viscosity and red cell aggregation. Via free radicals and and arachidonic acid derivatives

they may alter red cell membrane properties and thus modify red cell deformability. Free radicals increase both aggregability and rigidity of the red cells, while proteases mostly increase aggregation. Several cytokines released by monocytes trigger fibrinogen release by hepatocytes: IL6, leukemia inhibitory factor (LIF), and mostly oncostatine M (OSM) which induces a six-fold rise in fibrinogen production by hepatocytes. It is thus not surprising to notice that leukocyte activation, which is associated with release of such substances, is generally also characterized by a rise in plasma viscosity, red cell aggregability, and red cell rigidity [117]. These interactions between white cell activation and red cell rheology are currently being investigated extensively by the team of Baskurt [118–137,139]. On the whole, these studies strongly suggest that most of the hemorheologic profile of vascular or inflammatory diseases is explained by these white cell–red cell interactions [116].

3.3. Stress and rheology

By many mechanisms, stress is able to modify blood rheology. However, the whole mechanism is far to be clear. Fifteen years ago, Ehrly reported an experiment of videofilm-induced emotional stress in which the flow properties of blood were markedly modified by stress [120]. Some preliminary reports in depression also evidence a decrease in red cell deformability, mostly in endogenous depressive states [121]. As discussed above, there is not a complete agreement on the direct effects of norepinephrine and epinephrine on red blood cell deformability [96,97]. In the most recent studies, rather than simply reducing deformability, epinephrine appears to exert two opposite actions: it both induces formation of echinocytes and improves RBC deformability [97]. In fact, indirect effects of catecholamines and cortisol on circulating lipids may also explain an hemorheologic impairment during stress [121].

3.4. Fuel regulating hormones and the endocrine pancreas

It is interesting to notice that the pancreatic fuel-regulating hormones insulin [16,17,83–89], glucagon [90], somatostatin [91–94] and C-peptide [95] have all been reported to exert direct effects on red cell deformability. This suggests a physiological role of this hormonal axis in the regulation of blood rheology, consistent with the previously demonstrated circulatory actions of these hormones, and most particularly insulin which mediates vasodilation by direct stimulation of release of NO from endothelium, an effect which plays an important role in its fuel-regulatory actions [122].

3.5. Body composition: the adipose tissue

The adipose tissue now appears to be a true "endocrine gland" which synthesizes and releases a host of biologically active substances [123,124]. Several factors released by adipocytes may interfere with blood rheology. The most obvious, of course, are free fatty acids, but two important regulators of fibrinogen circulating levels, namely PAI-1 [125] and interleukin 6 [126], are also produced in significant amounts by adipocytes. This may explain why fibrinogen levels are positively correlated to fat mass [127], and why red cell aggregability is proportional to fat mass even within a physiological range in athletes [128]. This issue was recently studied by Yudkin [149] who performed a cross-sectional study in 107 nondiabetic subjects and observed that levels of C-reactive protein, and concentrations of the proinflammatory cytokines interleukin-6 and tumor necrosis factor-alpha, were related to all measures of obesity. These data suggest that adipose tissue is an important determinant of a low level, chronic inflammatory state as reflected by levels of interleukin-6, tumor necrosis factor-alpha, and C-reactive protein. Thus, in obesity

there is a low-level, chronic inflammatory state may induce insulin resistance and endothelial dysfunction and thus link the latter phenomena with obesity and cardiovascular disease. Although these authors did not measure rheologic parameters, it is likely that they are modified proportional to the inflammatory cytokines levels.

Another cytokine released by the adipose tissue is leptin. This fuel-regulating hormone circulates at high concentrations in the plasma of obese patients [130]. This hormone as been recently shown to stimulate proliferation and migration of myocytes in the vessel wall, and has thus been suggested to be a possible pathogenetic factor for atherogenesis [131]. We recently reported positive correlations between leptin and both plasma viscosity and RBC partial disaggregation threshold measured by laser backscattering. However, multivariate analysis indicated that the percentage of fat which is a major determinant of all these parameters was likely to explain all these correlations, so that a possible involvement of leptin in blood rheology remains unclear [132].

3.6. The growth-hormone IGF axis

The GH-IGF-I axis has major effects on body composition, fuel and fluid metabolism, which are likely to influence blood viscosity and red cell rheology [133]. Accordingly, in GH deficient adults the shift in lean/fat mass resulting from the GH insufficiency induces high plasma fibrinogen levels [134] and hemorheologic disturbances [135]. While GH deficiency is associated with impaired sweating, the enhanced GH response to exercise in trained athletes is correlated to sweating capacity and to the rise in plasma viscosity that occurs as a result of the associated increase in water loss [136]. Thus, GH via its effects on body fluids and body composition, appears to be rather beneficial for blood fluidity. By contrast, IGF-I which mediates most of GH anabolic actions and has receptors on erythrocytes has been found to positively correlate with blood viscosity, since high values of IGF-I are associated to lower red cell deformability and increased red cell aggregability [137]. The IGF-binding protein IGF-BP1, which physiologically traps IGF-I in order to moderate its action has a direct effect on erythrocyte progenitors and is negatively correlated with blood viscosity and positively correlated with the percentage of extracellular water in total body water. Since subjects in the upper tertile of IGF-BP1 compared to those in the lower had a higher erythrocyte deformability IGF-BP1 may also be a regulator of red cell rheology [138].

4. Hemorheology as a mirror of the metabolic/hormonal/inflammatory status of plasma in pathology

4.1. The case for cancer

In ovarian cancer there is a lower disaggregability of RBCs and a tendency to blood hyperviscosity compensated by a reduction of hematocrit [139–141]. A similar picture has been described in a series of 50 cancers of head and neck (compared to 80 controls) recently analyzed by Khan [141]. This author evidenced a rise in plasma viscosity, red cell aggregation, red cell rigidity and plasma fibrinogen. Another report by Ranade and coworkers [142] also evidenced abnormalities of blood rheology in head and neck cancers and found that plasma viscosity became frankly non-Newtonian in critical patients, perhaps related to alterations of plasma components by substances released by the cancer. Another study by Karabanov and coworkers [143] examined blood rheology in 163 patients with gastric cancer and found

abnormally high blood and plasma viscosity together with red blood cell hyperaggregation. Here again these changes were associated with an abnormal protein pattern.

We recently reported [141] that the hyperaggregation already described by previous investigators in ovarian cancer [139,140] is associated with a lower ability to dissociate erythrocyte aggregates. Actually, the exact biochemical mechanism of RBC hyperaggregation in cancer patients is not clear. In the paper of Khan [141], end-stage tumors were associated with a rise in whole blood viscosity which was assumed to be due to a specific (unknown) factor released by the tumor. This hypothesis of a specific tumor factor is in agreement with the findings of von Tempelhoff [140] who observed higher RBC aggregability in ovarian cancer compared to breast cancer. However, this investigator [140] observes that, in ovarian cancers, the highest plasma viscosity values are mostly due to increased plasma protein content (D-dimer and fibrinogen). Another hypothesis can be made if we consider that this hemorheologic pattern of an increased disaggregation threshold, associated to unchanged other aggregation parameters, has been described after experimental exposure of RBCs to oxygen free radicals [119]. Therefore, free radical generation that may occur, during inflammatory processes, as a consequence of leukocyte activation, could provide an alternative explanation for our finding. Other studies including markers of oxidant stress will be needed to clarify this point.

Regardless the mechanism which remains to be clarified, rheologic abnormalities in cancer appear to be related to the evolution and prognosis of the disease. There are several reports on larger series which strongly suggest that blood rheology may be a fair marker of evolutivity in several cancers [144,145], including the ovarian cancer [139]. Interestingly, treatment by chemotherapy improved this hemorheological pattern except when there were intercurrent thromboembolic events [146].

Rheologic alterations have also been reported to be predictive of deep venous thrombosis (DVT) in ovarian cancer [139,146]. The value of plasma viscosity before operation is predictive of further DVT, while the postoperatory value has no predictive value. It appears that, in ovarian cancer, a value of plasma viscosity below 1.34 mPa.s almost excludes the occurrence of a latter DVT [139]. In lung cancer, an algorithm including rheological parameters has been proposed by Skorniakov [145]. This algorithm for predicting thrombotic complications based on the minimal set of informative criteria appeared to be accurate in 75% of patients before surgery and 82.2% of patients on day 1 postoperation.

In women with ovarian cancer we also observed a correlation between hematocrit and corrected blood viscosity [141]. This correlation indicates that hematocrit is decreased in subjects with high factors of viscosity, so that apparent blood viscosity remains almost the same in controls and patients. This phenomenon of low hematocrit compensating a tendency to high viscosity is surely related to the homeostatic phenomenon of "*viscoregulation*" described above [109–114]. A reduction in hematocrit in most subjects is thus likely to be a regulatory response preventing to some degree the occurrence of an overt hyperviscosity syndrome. Interestingly, some literature suggests that this pattern of high plasma viscosity–low hematocrit is related to a higher risk of deep venous thrombosis [146].

4.2. The insulin resistance syndrome

Insulin resistance is a common metabolic abnormality that is associated with an increased risk of both atherosclerosis and type 2 diabetes [147]. The phenotype of insulin resistance includes a dyslipidemia characterized by an elevation of very low-density lipoprotein triglyceride, a reduction in high-density lipoprotein cholesterol, and the presence of small, triglyceride-enriched low-density lipoproteins. The underlying metabolic abnormality driving this dyslipidemia is an increased assembly and secretion of very low-density lipoprotein particles, leading to an increased plasma level of triglyceride. Hypertriglyceridemia, in turn, results in a reduction in the high-density lipoprotein level and the generation of small,

dense low-density lipoproteins; these events are mediated by cholesteryl ester transfer protein [148]. Fasting and postprandial concentration of triglycerides both depend on free fatty acids and insulinemia which are two separate and statistically independent determinants, indicating that hypertriglyceridemia results from defects in the ability of insulin to inhibit adipose tissue lipolysis [149]. In addition, hypertension, obesity, and a prothrombotic state are also integral components of the insulin resistance syndrome [148]. As extensively studied in our laboratory, fibrinogen levels are closely correlated to insulin resistance [150–152]. Not surprisingly, blood viscosity is also negatively correlated to insulin sensitivity [153–155]. A more precise analysis of this relationship indicates that while hyperaggregability of erythrocytes is rather related to the hyperinsulinism associated to insulin resistance, plasma viscosity is in fact directly related to the level of insulin sensitivity [156]. Accordingly, the treatment of insulin resistance by exercise training improves more specifically plasma viscosity, i.e., the parameter which is the most closely related to insulin sensitivity in these subjects [157,158].

5. Conclusion

The mechanisms reviewed above explain why so many situations, either physiological (diet, exercise) or pathological (diabetes, uremia) are associated with marked changes in blood rheology that may in turn modify micro- and macro-circulatory hemodynamics and the distribution of O_2 and fuels to tissues. Clearly, our knowledge of this question, despite the number of papers reviewed here, is far to be complete and many points remain to be studied. We think that a more extensive research on the influences of various humoral factors on blood rheology is needed in order to better understand how factors of blood rheology may represent a link between metabolic alteration and circulatory modifications either in pathology or in physiology.

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