

## Extended physiological functions for erythrocyte deformability and aggregation beyond regulation of oxygen delivery?

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### Abstract

An interpretative synthesis of all factors that have been shown to influence erythrocyte deformability and aggregation leads to postulate several regulatory axes in which blood rheology appears to be involved. One important regulatory loop involves 'viscoregulation' by erythropoietin, which governs the balance between red cell mass and blood fluidity and is a regulator of metabolism. Erythrocytes are also local regulators of water osmolality via the water-conducting channel aquaporin-1, so that when hyper or hypo-osmolality appears in a microvascular segment, red cell transit velocity decreases as a consequence of increased RBC rigidity, allowing this AQP1-driven exchange to completely restore iso-osmolality. Erythrocyte energy status is a major determinant of their rheologic properties, involving the release of ADP and ATP. Increasing values of glucose, cholesterol and triglycerides are associated with a decrease in red cell deformability while hormones regulating the flux of those substrates rather improve red cell rheology. Increase in circulating levels of GH and IGF1 are reported to be associated with moderately increased red cell rigidity and aggregation, while IGFBP1 may improve viscosity factors, modulating tissue supply of substrates needed for anabolism. An effect of sex hormones on red cell rheology may be involved in the regulation of fertility. Catecholamines also regulate during stress RBC rheology via  $\alpha$ - and  $\beta$ -adrenergic receptors.

**Keywords:** Erythrocyte deformability, metabolism, hormones, homeostasis, anabolism, eryptosis, stress

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### 1. Red cell rheology as an important physiological property of the Erythron

Over the last decades, the rheological properties of blood have been the matter of extensive research [1]. Mechanism and physiological relevance of red cell deformability [2] and aggregation [3] have been extensively studied. The passage of RBCs through microvessels is obviously crucial for transfer to tissues of oxygen which is necessary for life [4] but also of various chemical mediators whose vasoactive and antiadhesive properties aim at optimizing blood flow in tissues as a function of need [5]. It has been shown that RBCs deform and offload oxygen in gradually narrower and more hypoxic microvessels, so that this deformation, associated to hemoglobin deoxygenation, triggers the release of ATP and NO that can in turn regulate microvascular tone.

This integrated physiology of red cells which, put together, can be considered as a whole organ, has been termed the Erythron [6,7]. The Erythron comprises RBCs at all stages of development and is thus the organ responsible for oxygen (O<sub>2</sub>) transport, but also has a function of paracrine vasoregulation which adapts regional blood flow to O<sub>2</sub> exchanges in both the lung and the periphery [8].

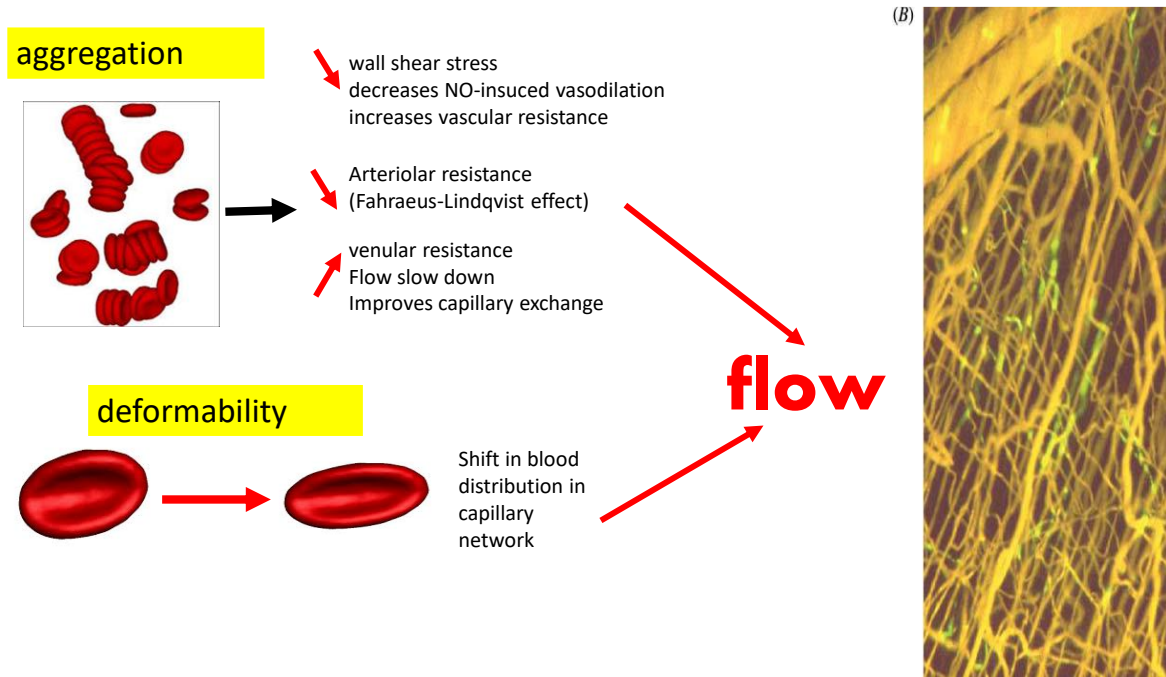


Fig. 1. Simplified cartoon summing up the effects of hemorheological alterations on circulation.

Classical studies on red cell deformability led to the concept that mammalian erythrocytes behave like fluid droplets, so that they undergo continuous deformation by deformation orientation, membrane tank treading, and cytoplasmic eddy flow, and that the red cell membrane behaves as a two dimensional elastoviscous fluid film [9]. Microscopic flow visualization of the process of red cell adaptation to flow shows that this fluid drop-like adaptation primarily depends on cytoplasm fluidity and surface-area-to-volume ratio, so that an excess of surface area allows strong deformations without an increase in surface area [10]. However, red cell deformation has recently been shown to be far more complex than previously assumed. Lanotte and co-workers [11] showed that RBCs undergo a wide variety of shape modifications during their deformation. As the shear rate increases, RBCs were first shown to tumble, then to roll, then they adopted the form of a tumbling stomatocyte, and finally above a threshold value of the viscosity contrast between plasma and cytosol they exhibited a variety of polylobed morphologies.

We recently pointed out another important aspect of this issue: the links between red cell rigidity and eryptosis [12]. As shown on Fig. 2 some cases of red cell stiffening occur during the process of programmed erythrocyte death (termed eryptosis) which is hardly reversible while other ones are physiological adaptations that are likely to be most of the time reversible.

Classical experiments using artificially stiffened RBCs demonstrate that a decrease in RBC deformability can markedly impair tissue perfusion [13]. Examples of very rigid RBCs, that are close to this experimental model, exist in human pathology in sickle cell disease [14]. In this case stiffened RBCs are able to induce vessel occlusion. However, most of the time, modifications of RBC deformability are less important and cells are still able to deform to some extent, and to transit in the microcirculation, but they are likely to pass only in the largest channels and thus to induce a capillary maldistribution of flow. Thus, beside “overtly abnormal blood rheology” (e.g. sickle cell crisis), there are many situations of “covertly abnormal” blood rheology [15], which do not result in vessel occlusion but are likely to induce alterations in tissue perfusion [16].

Red blood cell (RBC) aggregation is also a complex phenomenon [17]. It clearly exerts hemodynamic effects. Some of them (plasma skimming, Fåhræus Effect, microvascular hematocrit) that may improve rather than impede vascular blood flow [18], but via its effect on the intravascular velocity profile of red cells, its rise has been shown to decrease flow-related vasodilation and thus to impair blood flow [19].

A very important advance in our understanding of the circulatory effects of blood viscosity factors comes from the seminal works of the team of Marcos Intaglietta [20] which show that small increases in

blood viscosity in healthy individuals actually improve (and not impair!) cardiovascular function, due to the shear-induced release in vasodilatory molecules such as NO. These changes occur within the physiological range of variations of viscosity in the healthy population. This regulatory phenomenon may be impaired when endothelial function is deficient, and of course reaches a limit when increases in viscosity exceed the physiological range. This has led Sandro Forconi to propose a new important paradigm for hemorheology: As this investigator wrote, “While the traditional view postulates that an increased blood viscosity has invariably a negative impact on tissue perfusion and therefore should be considered as a risk factor (when not as a true disease), a more recent hypothesis has been formulated based on the observation that small increases in viscosity actually have vasodilatory effects, potentially improving tissue perfusion” [21].

Via NO production by the red cell itself or by the endothelium under the influence of RBC-induced shear, erythrocyte deformation may thus influence many important functions like blood distribution, but also angiogenesis, mitochondrial respiration, mitochondrial biogenesis, glucose uptake, muscle calcium homeostasis and thus muscle contractile properties and muscle force generation [22]. All this is likely to expand the range of functions regulated by red cell rheology.

Put together, all this information clearly indicates that the Erythron via its hemorheological properties exerts complex but important effects on the distribution of blood flow (and thus oxygen) in the body.

## 2. Erythrocytes as cells under influence

An impressive number of factors have been shown to influence erythrocyte deformability and aggregation [23]. They include physicochemical properties of the surrounding milieu such as pH, osmolality [24], divalent cations and albumin, blood lipids, and many hormones.

Recent reviews on this issue mention more than 30 factors and this number is surely underestimated [12, 25].

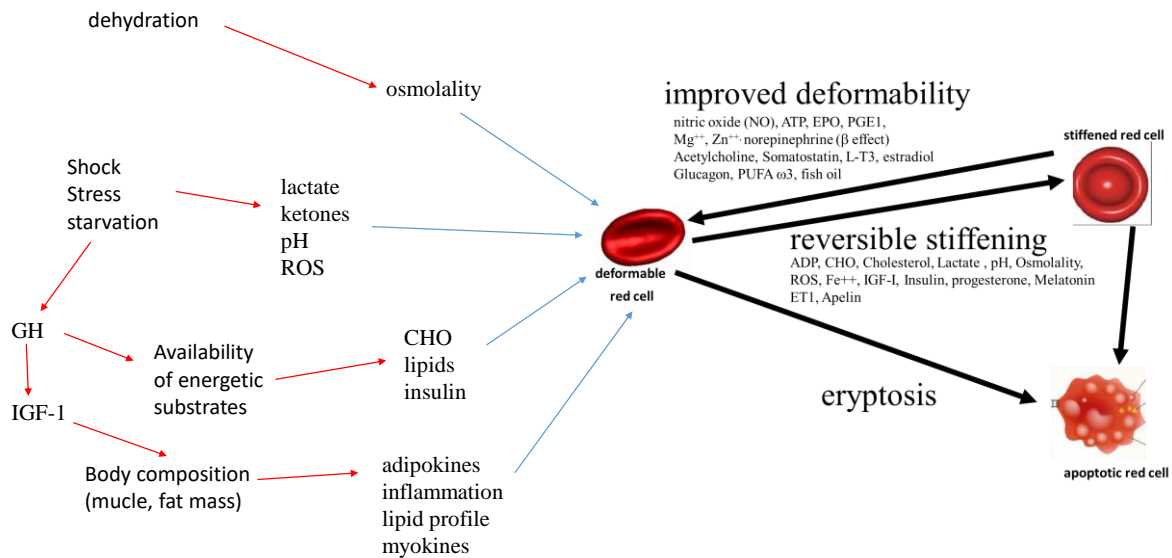


Fig. 2. Relationships between hormones, metabolism and blood rheology showing that several regulatory loops are involved in these interactions. Changes in red cell deformability may be regulatory reversible alterations or irreversible processes leading to red cell programmed death (eryptosis).

In this context we would emphasize the role of erythropoietin (EPO), a hormone released by the juxtaglomerular apparatus in the kidney’s nephrons, in response to hypoxia and which is inhibited by

increases in plasma viscosity. EPO stimulates erythrocyte development in the bone marrow. The team of W. Reinhardt has shown that a rise in plasma viscosity inhibits EPO and results in a decrease in RBC mass and hematocrit, thus representing a major mechanism of viscoregulation [26]. EPO also increases red cell deformability and decreases red cell aggregation [27] and exhibits antieryptotic properties [28]. Undoubtedly, EPO is one of the major regulators of blood rheology, involved in an important homeostatic loop which governs a very fine tuning of the balance between red cell mass and blood fluidity.

Interestingly, EPO is not only a regulator of red cell volume and blood viscosity, but is also a regulator of metabolism which enhances fat utilization and lowers the oxidation of carbohydrates during exercise [29]. Erythropoietin has been reported to increase in human's mitochondrial fatty acid oxidation capacity and myoglobin concentration in muscles [30] but this effect is not found by all authors [31]. On the whole we can conclude that this hormone improves the transport of oxygen and substrates to tissues via its erythropoietic and hemorheological effects, and also improves the oxidation, via the tricarboxylic acid cycle, of the fuels it delivers by the oxygen it also delivers.

### **3. Erythrocytes as local regulators of water osmolality**

A recent study by Sugie and co-workers pointed out that the water-conducting channel aquaporin-1 (AQP1), which is expressed on the red cell membrane, may drive a rapid water exchange between the red cell and the surrounding milieu resulting in an important volume change (up to 39%) [32]. Therefore erythrocytes can be compared to "micropumps" that regulate in situ local osmolality.

Since red cell deformability is strongly influenced by the osmolality of the surrounding milieu and dramatically decreases in both hyper or hypoosmolar ambience [12], it is likely that when hyper or hypoosmolality appears in a microvascular segment, red cell transit velocity decreases as a consequence of increased RBC rigidity, allowing this AQP1-driven exchange to completely restore isosmolality.

This novel property of the red cell is thus another homeostatic loop involving red cell rheology.

### **4. Erythrocyte rheological properties as regulators of energy metabolism: how many homeostatic loops?**

#### *4.1. Overall energy status*

Since erythrocytes need energy to undergo deformation, their energy status is a major determinant of their rheologic properties, as demonstrated by the gradual increase in the RBC rigidity which occurs during in vitro storage parallel to a decrease in intracellular ATP.

This energy status of the red cell is regulated by two homeostatic loops that involve purinergic signalling.

The first loop is the loop of stiffening. A decrease in RBC deformability inhibits the release of ATP [12] while stiffened erythrocytes release ADP, and ADP inhibits ATP release. This mechanism probably aims at maintaining RBC energy stores, but it is also likely to induce a self-potentiating loop resulting in RBC rigidification.

The second loop is the loop of deformability. When red cells become more deformable ATP release increases, and these well-deformable ATP-producing erythrocytes induce more NO production by the vessel wall, resulting into vasodilation and increased RBC deformability [33].

#### *4.2. Carbohydrate metabolism*

The relationships between erythrocyte rheologic properties and carbohydrate metabolism are complex. While initial studies did not evidence a clear effect of hyperglycemia on blood rheology, chronic hyperglycemia actually increases red cell rigidity above a threshold value of 9.05% glycated hemoglobin, indicating that the average blood glucose levels should remain above 200 mg/dL to result in a measurable decrease in red cell deformability [34].

Erythrocytes from diabetic patients incubated in high glucose concentrations became more aggregable and less deformable, due a change in the shape of RBCs whose perimeter-to-area ratio was increased [35].

This effect was observed in the RBCs from diabetic patients but not in the RBCs from healthy subjects. This question was also investigated by Shin [36], who observed significant hemorheological changes in red cells incubated with glucose. Both the deformability and aggregation of the erythrocytes decreased in a dose- and time-dependent manner. These authors interpreted these hemorheological modifications as a consequence of the glucose-induced (auto)oxidation and glycation of the erythrocytes.

RBC rigidity is also positively correlated with carbohydrate intake in trained athletes, in whom positive correlations are evidenced between caloric (and CHO) intake and both RBC rigidity and aggregability [37].

Lactate generated by carbohydrate metabolism upstream in the tricarboxylic acid cycle and released in blood during hypoxia or exercise, decreases erythrocyte deformability. Above a threshold concentration of 4 mmol/L, but in highly trained endurance athletes, it has the opposite effect and improves erythrocyte deformability [38].

Ketone bodies, an alternative fuel used by tissues in some physiological situations like starvation, or ketogenic diets, are *in vivo* associated with a decrease in red cell deformability [39].

#### *4.3. Glucoregulatory hormones*

The main hormone of glucose homeostasis, insulin, has receptors on the red cell membrane and its binding to these receptors activates intracellular pathways. Insulin's effects on erythrocyte deformability have been reported for 40 years and are well confirmed and described today [12]. The influence of insulin on erythrocyte rheology seems to be mediated by an effect on the molecular composition of the lipid membrane bilayer and its microviscosity, together with modifications of the function of membrane Na/K ATPase. Insulin improves erythrocyte deformability below a threshold value of supraphysiological levels which, on the opposite, decrease in RBC deformability. These effects of insulin are probably also related to a regulatory effect of insulin on RBC intracellular ATP concentrations [40].

The pancreatic hormone C-peptide co-secreted with insulin by the beta cell has vasoregulatory effects due to a stimulatory action of eNOS in diabetics, and increases RBC membrane fluidity [41]. Using laser-diffractoscopy a huge improvement in erythrocyte deformability could be observed after C-peptide administration in erythrocytes of type 1 diabetic patients. Inhibition of the Na<sup>+</sup>K<sup>+</sup>ATPase completely abolished the effect of C-peptide on erythrocyte deformability [42].

Another important hormone involved in the regulation of fuel metabolism, glucagon, which exerts an action opposite to that of insulin, was first reported to *in vitro* decrease RBC deformability [43] and then to *in vivo* increase it, resulting in a decrease in blood viscosity and an increase in the blood flow [44]. This issue remains subject of argument [12].

Somatostatin is also a regulatory hormone that acts within the pancreas and is also released into the general circulation. It has been shown to improve red cell deformability [45].

Actually, all those pancreatic hormones: insulin [46] C-peptide [42], somatostatin [47] and glucagon [48] are vasodilator substances that decrease vascular resistance and modify blood flow distribution. Their hemorheological action can be one of the mechanisms of their flow-regulating effect.

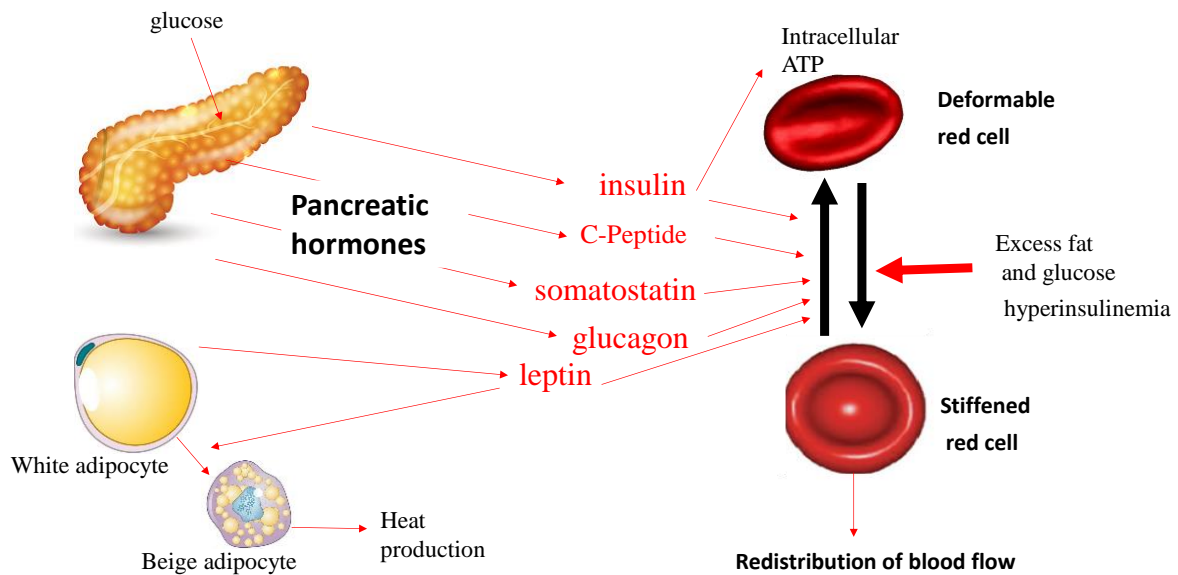


Fig. 3. An attempt to interpret the relationship between blood rheology and factors of energetic metabolism. On the whole, increasing values of glucose, cholesterol and triglycerides are associated with a decrease in red cell deformability while hormones regulating the flux of energetic substrates improve red blood cell rheology.

#### 4.4. Adipose stores and fat metabolism

It is well established that circulating lipids are associated with impaired erythrocyte rheology. Increased levels of either LDL cholesterol and triglycerides are associated with an impairment in RBC deformability while HDL-cholesterol is positively associated with red cell deformability [49]. By contrast omega-3 fatty acids decrease blood viscosity, fibrinogen; and erythrocyte rigidity [50].

Erythrocyte aggregation is increased in obesity [51] and a decrease in adipose stores induced by bariatric surgery reverses this hyper aggregation [52]. Actually, fatness by its own is associated with increased red cell aggregation, while abdominal fat increases blood viscosity due to a rise in hematocrit, and overall body size is associated with increased plasma viscosity and red cell rigidity [53]. In obesity, the increase in RBC aggregation seems to be to some extent related to low grade inflammation [54] but it is important to point out that RBC aggregation is correlated to fat mass even in normal weight individuals and not only in obesity [55]. This leads to the concept that the mass of adipose stores, even within a physiological range, is a determinant of the extent of RBC aggregation.

In elite athletes, lipid intake (g/kg/day) is negatively correlated with the RBC disaggregability threshold while low calorie intake is associated with lower RBC disaggregability [56]. In fact, the relationships between nutrition and blood rheology seem to be not exactly similar in sedentary and in physically active individuals and this relationship is no longer found in sedentary subjects [37].

The ability to oxidize more lipids during exercise is found to be associated with improved RBC deformability [57]. It has been assumed that this relationship can be explained by the effects of endurance training on lipid oxidation which may in turn modify both lipid metabolism and free radical generation, thus influencing RBC rheology.

An important fuel-regulating hormone, leptin, which is released by the adipose tissue, improves red cell deformability via a NO- and cGMP-dependent mechanism [58]. We recently reported that it is not only associated with increased red cell deformability but also with increased aggregation [12]. Whether leptin mediates the relationship between fat mass and RBC aggregation remains to be evaluated but it is important to point out that this hormone related to the volume fat stores has an impact on RBC rheology. The major

physiological function of leptin is to signal states of negative energy balance and decreased energy stores. but in the context of obesity, a defective thermoregulatory response is associated with impaired leptin signalling within the hypothalamus, leading to the concept of a role of leptin in the regulation of adaptive thermogenesis. Therefore, leptin can be considered as a thermolipokine [59]. Leptin can stimulate thermogenesis in brown adipose tissue and the browning of white adipose tissue [60].

There is strong evidence that leptin is able to dilate blood vessels via both nitric oxide-dependent and nitric oxide-independent mechanisms [61]. Under physiological conditions leptin induces endothelium-dependent vasorelaxation by stimulating nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Leptin activates endothelial NO synthase (eNOS) through a mechanism involving AMP-activated protein kinase (AMPK) and protein kinase B/Akt. Actually, while the effect of leptin in healthy subjects is mostly to promote vasodilation, hyperleptinemia in obese subjects seems to gradually dysregulate blood pressure control by deteriorating endothelial functions. When metabolic syndrome develops, the contribution of EDHF to the haemodynamic effect of leptin becomes inefficient. Resistance to the vasodilatory effects of leptin may contribute to the development of arterial hypertension owing to unopposed stimulation of the sympathetic nervous system by this hormone [62].

#### *4.5. Hemorheological effects of energetic substrates vs their regulating hormones*

To sum up the literature reviewed in this chapter, it appears that increasing circulating levels of both carbohydrates and fat impair erythrocyte rheology, and more precisely increase red cell rigidity and red cell aggregation.

By contrast the effects of insulin, C peptide, leptin, and presumably glucagon, are to improve blood fluidity and all those hormones have vasodilatory properties. When supraphysiological levels of some of these hormones are reached, there may be a reversal of this effect and they may impair blood rheology, as reported with insulin. This is logical since the function of those hormones is to increase the flux of energetic substrates, either inward to or outward from the tissues.

In this context, beside the impact of those hormones on the endothelium, their hemorheological effects are likely to participate to the fine tuning of this homeostasis.

### **5. Erythrocyte rheology and anabolic functions**

The growth hormone (GH) - insulin-like growth factor I (IGF-I) axis is the major hormonal system positively controlling anabolism and cell growth in mammals.

Short term administration of recombinant human growth hormone induces a increase in red cell aggregation. In thirty young healthy males 7 days administration of human rhGH (compared to placebo) resulted in a significant increase in erythrocytes aggregation index post injection (day 8), parallel to an increase in serum IGF-I [63]. Hemorheological effects of growth hormone may be at least in part mediated by their effects on lipid metabolism and fluid homeostasis. For example, the GH response to exercise is correlated with extracellular water volume [64].

By contrast in growth hormone deficient (GHD) adults there is no evidence for an impairment of blood rheology other than that related to increased fat mass. There is only a nonsignificant tendency for plasma viscosity to be higher in female but not male GHDs [65].

Insulin-like growth factor I (IGF-I) has been associated with increased blood viscosity. When IGF-I values are within the upper quintile (>340 ng/ml) IGF-I appears to unfavourably affect blood rheology. Crude IGF-I levels exhibit a borderline correlation with red cell rigidity index "Tk" [64]. This relationship was confirmed in another study reporting correlations between IGF-I and both whole blood viscosity and red cell rigidity "Tk" [66]. The ratio IGF1/IGFBP3 which reflects free circulating IGF-I is correlated with red cell aggregability measured with the Myrenne "M" and the SEFAM-AFFIBIO aggregometer index S60.

Therefore, values of IGF-I within the upper quintile are associated with an impairment of blood fluidity, possibly due to a direct effect of IGF-I on red cell deformability and aggregability.

Another potential candidate for such a rheological effect of the GH-IGF axis is insulin-like growth factor binding protein-1 (IGF-BP1) which is increased in trained people and correlated to fitness: IGF-BP1 is elevated in patients with polycythaemia vera and stimulates erythroid burst formation in vitro. In

professional soccer players, IGF BP1 was negatively correlated with blood viscosity at high shear rate and positively correlated with the percentage of extracellular water in total body water. Therefore, beside GH and IGF-I, IGF-BP1, which is reported to act on erythroid progenitors, also exhibits statistical relationships with blood fluidity and erythrocyte flexibility that may suggest a possible physiological role in improving blood rheology [66].

Therefore, on the whole, increase in circulating levels of GH and IGF1 are reported to be associated with moderately increased red cell rigidity and aggregation, while IGFBP1 may improve viscosity factors. The physiological meaning of these findings is not clear.

## **6. Erythrocyte rheology and reproductive functions**

Sex Hormones appear to modify red cell rheologic properties. Estradiol levels have been reported to be correlated with RBC rigidity, while progesterone is associated with increased RBC deformability, so that RBC deformability is lower during the follicular phase compared than in the luteal phase. Hormonal treatment by estrogens impairs erythrocyte deformability by increasing membrane rigidity and decreases RBC aggregation. This should be interpreted under the light of other recent works which show that estrogens increase NO synthesis and release by endothelial cells. Isosorbide mononitrate as nitric oxide (NO) donors has been shown to improve ovulation and pregnancy rates in an ovulatory woman with polycystic ovary syndrome [67]. Actually, this issue of vasodilatory factors improving ovulation remains controversial [68]. On the other hand, estrogen and progesterone are also associated with increased eryptosis. Another sex hormone, dehydroepiandrosterone sulphate, also decreases red cell deformability [12].

What could be the physiological relevance of these findings? The physiological rise in estradiol during follicular phase is well known to be the hormonal event that triggers ovulation. It is known that estradiol increase triggers a midcycle surge of LH inducing interconnected networks of signaling cascades aiming at the rupture of the follicle and the release of the oocyte. During this event granulosa and theca cells release not only steroids, but also prostaglandins, chemokines, and cytokines, which are also mediators of inflammation [69]. Follicular hyperaemia was observed 30 min after the LH surge and then vasomotion can be observed. A gradual decline in the apical blood flow results in the appearance of an avascular area over the top of the apex [70]. Capillary permeability of the ovarian blood-follicle barrier is modulated by gonadotropin, mainly through increased numbers of large pores, similar to a classical inflammatory response [71].

In this context a moderate decrease in RBC deformability associated with a lower red cell aggregation and an increased NO release may play a role in the alterations in microcirculatory blood flow in the ovary.

After ovulation, the follicle undergoes rapid angiogenesis and functional differentiation of granulosa and theca cells [69]. Whether an improvement in red cell deformability induced by progesterone can participate to this process is not clear.

Thus, the effect of sex hormones on red cell rheology may be involved in the very complex mechanism of ovulation and thus play a role in the regulation of fertility.

## **7. Blood rheology and stress**

In 1936 Hans Selye [1907 – 1982] introduced the concept of a "General Adaptation Syndrome", as a neuro-hormonal response ("fight or flight response") aiming at maintaining the homeostasis in the body submitted to an unusual situation. In addition Selye discovered that even if one's body wants to control or reduce the stress, it may reach a limit, even more if the body is exposed to the stressor continuously. More than eighty years later this concept remains fully valid. Psychological and physiological stressors have been shown to stimulate biological stress responses including cortisol release via the hypothalamus–pituitary–adrenal (HPA) axis [72].

Not surprisingly, stress induces hemorheological modifications. A pioneering study was presented by A. Ehrly [73] evidencing a rise in blood and plasma viscosity after a video film-induced emotional stress. In this case hematocrit remained unchanged during the experiment. The authors concluded that "these observations could lead to new aspects of the pathophysiology of acute myocardial infarction initiated by



severe emotional stress".

More recently, as a consequence of the Fukushima disaster (11 March 2011), a hyperviscosity syndrome was evidenced among evacuees who survived this earthquake and tsunami followed by massive nuclear irradiation, that resulted into approximately 18,500 deaths. A chronic increase in RBC, Hb levels and hematocrit levels was reported 4 years after the earthquake [74]. Other reported effects of this evacuation after a major disaster were hypertension, diabetes, atrial fibrillation, hypo-high-density lipoprotein cholesterolemia, and obesity.

An increased incidence of cardiovascular events was also observed following another similar event, the Hanshin-Awaji earthquake and was previously reported to be associated with the elevated hematocrit [75]. It was assumed that stress-induced increase in red blood cell concentration and hematocrit leads to thrombosis [76].

On the whole, both acute and chronic stress situations induce a syndrome of blood hyperviscosity which seems to be associated with a higher risk of thrombotic events.

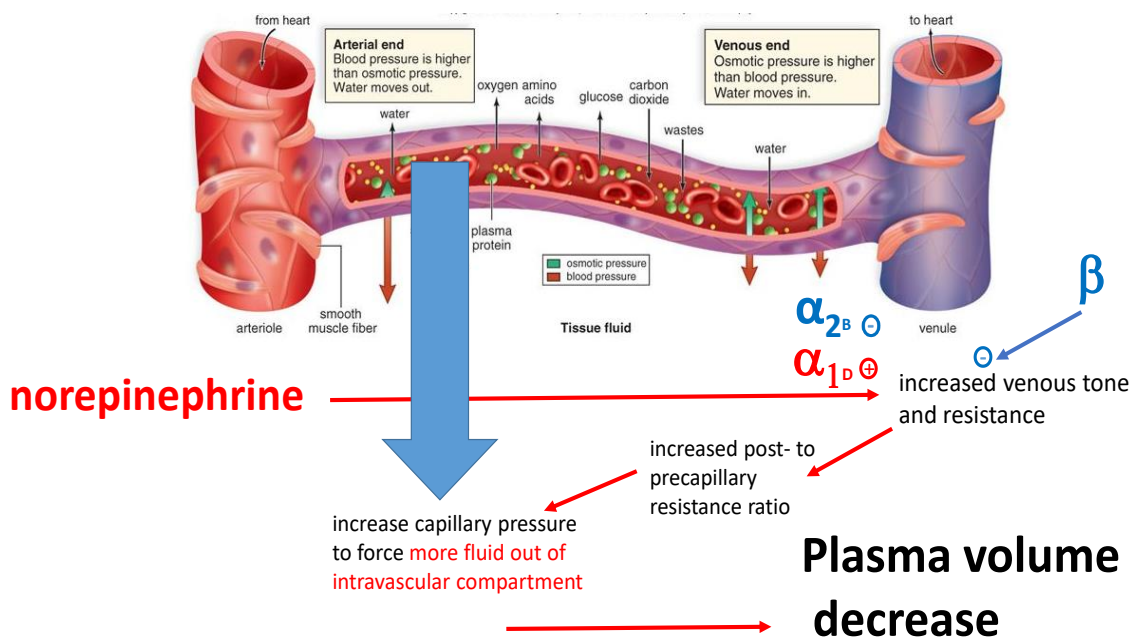


Fig. 4. Role of norepinephrine via alpha-1 signalling in stress-induced hyperviscosity.

An important regulator axis acting on RBC rheology may be catecholamines, which circulate in blood and act on RBCs via specific  $\alpha$ - and  $\beta$ -adrenergic receptors for regulating cell volume and ion transport. The first studies in the late eighties by Pfafferoth and Volger indicated that in vitro norepinephrine and isoprenaline reduced RBCs deformability [77]. More recently, a more complicated picture emerged from the works of Hilario, Saldanha and Martins-Silva who demonstrated in human RBCs that although epinephrine is able to induce the formation of echinocytes, it also improves RBC deformability [78]. This is in line with the reported anti-eryptotic effect of catecholamines and with the role of those hormones which are beneficial on the short term for body's adaptation to an unusual stress. In trouts where RBCs are nucleated, catecholamines induce a dramatic increase in cell volume as a result of an accumulation of sodium and chloride due to activation of an amiloride-sensitive, cyclic AMP-dependent  $\text{Na}^+\text{-H}^+$  exchanger allowing  $\text{Na}^+$  to enter in exchange for internal  $\text{H}^+$ . At the same time RBC deformability is improved (despite the increase in cell volume). Both RBC fluidification and activation of this ionic exchange are likely to be an adaptive response to hypoxia which results in an increased oxygen-carrying capacity of RBCs. Literature, however suggests that that epinephrine may improve RBC deformability [79], presumably via  $\beta$ -adrenergic receptors while there is apparently no effect of either  $\alpha_1$ - and  $\alpha_2$ -receptor agonists [80]. It was also shown that RBCs incubated with epinephrine and isoproterenol, resulted in significant changes of

deformability, by 10 and 30%, respectively. This is consistent with the other classical effects of catecholamines mediated by  $\beta$ -adrenergic receptors (vasodilation, increased cardiac output, etc.) that all lead to increase blood flow. The team of Alexei Muravyov has extensively studied the effect of catecholamines on the rheological properties of the human RBC, showing that the effect of these hormones on RBC deformability are mostly under the control of intracellular  $Ca^{2+}$  regulating pathways. In contrast with this positive effect of catecholamines in physiological conditions on RBC deformability, increased viscosity and decreased RBC deformability were observed in untreated pheochromocytoma [12].

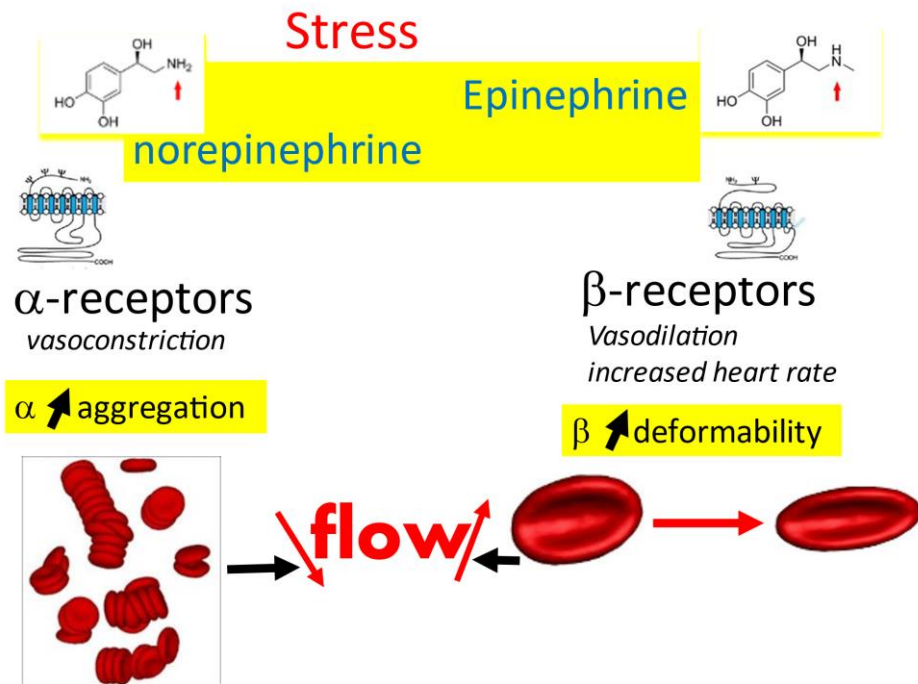


Fig. 5. Effects of catecholamines on erythrocyte rheology

Dabhar recently proposed “the stress spectrum model” which describes stress as a constellation of events, in response to a stimulus (stressor), that activates the fight-or-flight systems in the body. The duration of a physiological response to stress is the critical determinant of its effects on immune function and health. Acute stress generally results in activation of mechanisms that include enhancement of immune function while chronic stress results in health-averse conditions that result in dysregulation or suppression of immune function. [72, 81].

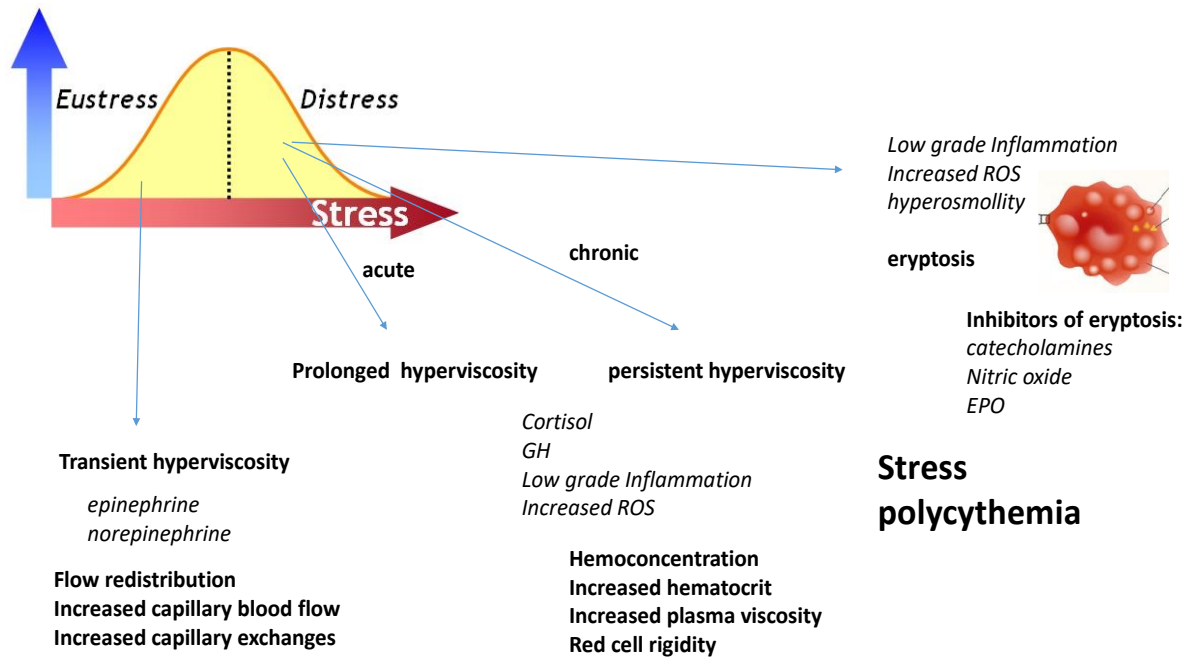


Fig. 6. Different stages of stress-induced hyperviscosity interpreted according to Dabhar's "stress spectrum model".

## 8. Conclusion

The aim of this paper is to propose an interpretative synthesis of the very wide body of literature on the interactions between metabolism, hormones, and hemorheology, that we reviewed previously several times [12, 23, 25]. Clearly, this chapter of physiology despite the number of papers evidencing interactions remains poorly understood, and we would want to stimulate research in this area.

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## References

- [1] Schmid-Schönbein, H., 1981. Blood rheology and physiology of microcirculation. *La Ricerca in Clinica e in laboratorio* 11, 13-33.
- [2] Huisjes, R. Bogdanova, A. van Solinge, WW., Schiffelers, RM. Kaestner, L. van Wijk, R., 2018. Squeezing for life – properties of red blood cell deformability. *Frontiers in Physiology* 9, 656.
- [3] Baskurt, OK. Meiselman, HJ., 2013. Erythrocyte aggregation: basic aspects and clinical importance. *Clinical Hemorheology and Microcirculation* 53, 1-2, 23-37.
- [4] Crystal, GJ. Pagel, PS., 2020. The physiology of oxygen transport by the cardiovascular system: evolution of knowledge. *Journal of Cardiothoracic and Vascular Anesthesia* 34, 5, 1142-1151.
- [5] McMahon, TJ., 2019. Red blood cell deformability, vasoactive mediators, and adhesion. *Frontiers in Physiology* 10, 1417.
- [6] Kaushansky, K. Lichtman MA. Beutler, E. Kipps, TJ. Seligsohn, U. Prchal, JT., 2010. *Williams Hematology* 8e ed: McGraw-Hill Professional Publishing Lincoln, Lincolnshire, UK.
- [7] Rogers, S. Doctor, A., 2020. Red blood cell dysfunction in critical illness. *Critical Care Clinics* 36(2), 267-292.
- [8] Doctor, A. Stamler, JS., 2011. Nitric oxide transport in blood: a third gas in the respiratory cycle. *Comprehensive Physiology* 1, 1, 541-568.
- [9] Schmid-Schönbein, H. Gaehtgens, P. Fischer, T. Stöhr-Liesen, M., 1984. Biology of red cells: non-nucleated erythrocytes as fluid drop-like cell fragments. *International Journal of Microcirculation: Clinical and Experimental* 3,

2, 161-196.

- [10] Schmid-Schönbein, H. Gaehtgens, P., 1981. What is red cell deformability? *Scandinavian Journal of Clinical and Laboratory Investigation* 156, 13-26.
- [11] Lanotte, L. Mauer, J. Mendez, S. Fedosov, DA. Fromental, JM. Claveria, V. Nicoud, F. Gompper, G. Abkarian, M., 2016. Red cells' dynamic morphologies govern blood shear thinning under microcirculatory flow conditions. *Proceedings of the National Academy of Sciences* 113, 13289-13294.
- [12] Brun, JF. Varlet-Marie, E. Myzia, J. Raynaud de Mauverger, E. Pretorius, E., 2021. Metabolic influences modulating erythrocyte deformability and eryptosis. *Metabolites* 12, 1, 4.
- [13] Simchon, S. Jan, KM. Chien, S., 1987. Influence of reduced red cell deformability on regional blood flow. *American Journal of Physiology* 253(4 Pt 2), H898-903.
- [14] Connes, P. Alexy, T. Detterich, J. Romana, M. Hardy-Dessources, MD. Ballas, SK., 2015. The role of blood rheology in sickle cell disease. *Blood Reviews* 30, 2, 111-118.
- [15] Schmid-Schönbein, H. Teitel, P., 1987. In vitro assessment of "covertly" abnormal blood rheology: critical appraisal of presently available microrheological methodology. A review focusing on diabetic retinopathy as a possible consequence of rheological occlusion. *Clinical Hemorheology and Microcirculation* 7, 203-238.
- [16] Barshtein, G. Ben-Ami, R. Yedgar, S., 2007. Role of red blood cell flow behavior in hemodynamics and hemostasis. *Expert Review of Cardiovascular Therapy* 5, 4, 743-752.
- [17] Stoltz, JF. Senger, B. Voegel, JC. Delamaire, M. Schaaf, P. Boisseau, M., 1995. Aspects rhéologiques et biophysiques de l'adhésion et de l'agrégation cellulaire : importance en hémorhéologie. *Journal des Maladies Vasculaires* 20, 4, 247-251.
- [18] Schmid-Schönbein, H., 1976. Microrheology of erythrocytes, blood viscosity, and the distribution of blood flow in the microcirculation. *International Review of Physiology* 9, 1-62.
- [19] Yalcin, O. Uyklu, M. Armstrong, JK. Meiselman, HJ. Baskurt, OK., 2004. Graded alterations of RBC aggregation influence in vivo blood flow resistance. *American Journal of Physiology-Heart and Circulatory Physiology* 287, 6, H2644-H2650.
- [20] Salazar Vázquez, BY. Martini, J. Chávez Negrete, A. Cabrales, P. Tsai, AG. Intaglietta, M., 2009. Microvascular benefits of increasing plasma viscosity and maintaining blood viscosity: counterintuitive experimental findings. *Biorheology* 46, 3, 167-179.
- [21] Forconi, S. Wild, P. Munzel, T. Gori, T., 2011. Endothelium and hyperviscosity. *Clinical Hemorheology and Microcirculation* 49, 1-4, 487-491.
- [22] Jones, AM., 2014. Dietary nitrate supplementation and exercise performance. *Sports Medicine* 44, 1, S35-S45.
- [23] Brun, JF., 2002. Hormones, metabolism and body composition as major determinants of blood rheology: potential pathophysiological meaning. *Clinical Hemorheology and Microcirculation* 26, 2, 63-79.
- [24] Koutsouris, D. Delatour-Hanss, E. Hanss, M., 1985. Physico-chemical factors of erythrocyte deformability. *Biorheology* 22, 119-132.
- [25] Brun, JF. Varlet-Marie, E. Richou, M. Mercier, J. Raynaud de Mauverger, E., 2018. Blood rheology as a mirror of endocrine and metabolic homeostasis in health and disease1. *Clinical Hemorheology and Microcirculation* 69, 1-2, 239-265.
- [26] Reinhart, WH., 2001. Molecular biology and self-regulatory mechanisms of blood viscosity: A review. *Biorheology* 38, 203-212.
- [27] Muravyov, AV. Cheporov, SV. Kislov, NV. Bulaeva, SV. Maimistova, AA., 2010. Comparative efficiency and hemorheological consequences of hemotransfusion and epoetin therapy in anemic cancer patients. *Clinical Hemorheology and Microcirculation* 44, 115-123.
- [28] Vota, DM. Maltaner, RE. Wenker, SD. Nesse, AB. Vittori, DC., 2013. Differential erythropoietin action upon cells induced to eryptosis by different agents. *Cell Biochemistry and Biophysics* 65, 145-157.
- [29] Caillaud, C. Connes, P. Ben Saad, H. Mercier, J., 2015. Erythropoietin enhances whole body lipid oxidation during prolonged exercise in humans. *Journal of Physiology and Biochemistry* 71(1), 9-16.
- [30] Guadalupe-Grau, A. Plenge, U. Helbo, S. Kristensen, M. Andersen, PR. Fago, A. Belhage, B. Dela, F. Helge, JW., 2015. Effects of an 8-weeks erythropoietin treatment on mitochondrial and whole body fat oxidation capacity during exercise in healthy males. *Journal of Sports Sciences* 33, 6, 570-578.
- [31] Risikesan, J. Nellemann, B. Christensen, B. Jørgensen, JOL. Nielsen, S., 2020. No effect of 10 weeks erythropoietin treatment on lipid oxidation in healthy men. *Endocrine Connections* 9, 12, 1148-1155.
- [32] Sugie, J. Intaglietta, M. Sung, LA., 2018. Water transport and homeostasis as a major function of erythrocytes. *American Journal of Physiology - Heart and Circulatory Physiology* 314, H1098-H1107.
- [33] Lockwood, SY. Erkal, JL. Spence, DM., 2014. Endothelium-derived nitric oxide production is increased by ATP released from red blood cells incubated with hydroxyurea. *Nitric Oxide* 38, 1-7.
- [34] Li, Q. Yang, LZ., 2018. Hemoglobin A1c level higher than 9.05% causes a significant impairment of erythrocyte deformability in diabetes mellitus. *Acta Endocrinologica (Bucharest)* 14, 1, 66-75.

- [35] Babu, N. Singh, M., 2004. Influence of hyperglycemia on aggregation, deformability and shape parameters of erythrocytes. *Clinical Hemorheology and Microcirculation* 31, 273-280.
- [36] Shin, S. Ku, YH. Suh, JS. Singh, M., 2008. Rheological characteristics of erythrocytes incubated in glucose media. *Clinical Hemorheology and Microcirculation* 38, 3, 153-161.
- [37] Varlet-Marie, E. Guiraudou, M. Fédou, C. Raynaud De Mauverger, E. Durand, F. Brun, JF., 2013. Nutritional and metabolic determinants of blood rheology differ between trained and sedentary individuals. *Clinical Hemorheology and Microcirculation* 55, 39–54.
- [38] Varlet-Marie, E. Brun, JF., 2004. Reciprocal relationships between blood lactate and hemorheology in athletes: Another hemorheologic paradox? *Clinical Hemorheology and Microcirculation* 30, 3,4, 331-337.
- [39] Peyreigne, C. Bouix, D. Benhaddad, AA. Raynaud, E. Perez-Martin, A. Mercier, J. Brun, JF., 1999. Hemorheologic effects of a short-term ketogenic diet. *Clinical Hemorheology and Microcirculation* 21, 147-153.
- [40] Aursnes, I. Dahl-Jørgensen, K. Hanssen, KF., 1986. ATP-concentrations in erythrocytes influenced by insulin levels in plasma. *Clinical Hemorheology and Microcirculation* 12, 6, 429-433.
- [41] Forst, T. De La Tour, DD. Kunt, T. Pflützer, A. Goitom, K. Pohlmann, T. Schneider, S. Johansson, BL. Wahren, J. Lobig, M. Engelbach, M. Beyer, J. Vague, P., 2000. Effects of proinsulin C-peptide on nitric oxide, microvascular blood flow and erythrocyte Na<sup>+</sup>,K<sup>+</sup>-ATPase activity in diabetes mellitus type I. *Clinical Science* 98, 283-290.
- [42] Forst, T, Kunt, T., 2004. Effects of C-peptide on microvascular blood flow and blood hemorheology. *Experimental Diabetes Research* 5, 1, 51-64.
- [43] Valensi, P. Gaudey, F. Attali, JR., 1986. La déformabilité érythrocytaire est réduite par le glucagon. *Diabetes & Metabolism* 12, 281.
- [44] Komatsu, R. Tsushima, N. Matsuyama, T., 1997. Effects of glucagon administration on microcirculation and blood rheology. *Clinical Hemorheology and Microcirculation* 17, 271-277.
- [45] Brun, JF. Rauturier, M. Ghanem, Y. Orsetti, A., 1991. In vitro effects of somatostatin on red cell filterability measured by three methods. *Journal des Maladies Vasculaires* 16, 49-52.
- [46] Bergman RN., 2003. Insulin action and distribution of tissue blood flow. *Journal of Clinical Endocrinology and Metabolism* 88, 10, 4556-4458.
- [47] Savi, L. Cardillo, C. Bombardieri, G., 1985. Somatostatin and peripheral blood flow in man. *Angiology* 36, 511-515.
- [48] Farah, AE., 1983. Glucagon and the circulation. *Pharmacological Reviews* 35, 3, 181-217.
- [49] Radosinska, J. Vrbjar, N., 2016. The role of red blood cell deformability and Na,K-ATPase function in selected risk factors of cardiovascular diseases in humans: focus on hypertension, diabetes mellitus and hypercholesterolemia. *Physiological Research* 65, 1, S43-54.
- [50] Simopoulos, AP., 1991. Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition*. 54, 3, 438-463.
- [51] Gyawali, P. Richards, RS. Hughes, DL. Tinley, P., 2014. Erythrocyte aggregation and metabolic syndrome. *Clinical Hemorheology and Microcirculation* 57, 1, 73-83.
- [52] Wiewiora, M. Picuch, J. Glück, M. Slowinska-Lozynska, L. Sosada, K., 2015. The effects of weight loss surgery on blood rheology in severely obese patients. *Surgery for Obesity and Related Diseases*. 11, 6, 1307-1314.
- [53] Guiraudou, M. Varlet-Marie, E. Raynaud de Mauverger, E. Brun, JF., 2013. Obesity-related increase in whole blood viscosity includes different profiles according to fat localization. *Clinical Hemorheology and Microcirculation* 55,1, 63-73.
- [54] Samocha-Bonet, D. Lichtenberg, D. Tomer, A. Deutsch, V. Mardi, T. Goldin, Y. Abu-Abeid, S. Shenkerman, G. Patshornik, H. Shapira, I. Berliner, S., 2003. Enhanced erythrocyte adhesiveness/aggregation in obesity corresponds to low-grade inflammation. *Obesity Research* 11,3, 403-407.
- [55] Brun, JF. Varlet-Marie, E. Cassan, D. Raynaud de Mauverger, E., 2011. Blood rheology and body composition as determinants of exercise performance in female rugby players. *Clinical Hemorheology and Microcirculation* 49,1-4, 207-214.
- [56] Gaudard, A. Varlet-Marie, E. Bressolle, F. Mercier, J. Brun JF., 2004. Nutrition as a determinant of blood rheology and fibrinogen in athletes. *Clinical Hemorheology and Microcirculation* 30,1, 1-8.
- [57] Brun, JF. Varlet-Marie, E. Cassan, D. Manetta, J. Mercier, J., 2004. Blood fluidity is related to the ability to oxidize lipids at exercise. *Clinical Hemorheology and Microcirculation* 30, 3-4, 339-343.
- [58] Tsuda, K. Kimura, K. Nishio, I., 2002. Leptin improves membrane fluidity of erythrocytes in humans via a nitric oxide-dependent mechanism: an electron paramagnetic resonance investigation. *Biochemical and Biophysical Research Communications* 297, 672–681.
- [59] Genchi, VA. D'Oria, R. Palma, G. Caccioppoli, C. Cignarelli, A. Natalicchio, A. Laviola, L. Giorgino, F. Perrini, S., 2021. Impaired leptin signaling in obesity: is leptin a new thermolipokine? *International Journal of Molecular Sciences* 22, 12, 6445.
- [60] Seoane-Collazo, P. Martínez-Sánchez, N. Milbank, E. Contreras, C., 2020. Incendiary Leptin. *Nutrients* 12(2),

472.

- [61] Leung, YM. Kwan, CY., 2008. Dual vascular effects of leptin via endothelium: hypothesis and perspective. *Chinese Journal of Physiology* 51,1, 1-6.
- [62] Bełtowski, J., 2012. Leptin and the regulation of endothelial function in physiological and pathological conditions. *Clinical and Experimental Pharmacology and Physiology* 39, 2, 168-178.
- [63] Ramos, SB. Brun, JF. Gray, B. Rogerson, S. Weatherby, RP. Tajouri, L. Marshall-Gradisnik, SM., 2011. The effects of short-term recombinant human growth hormone (rhGH) on blood rheology in healthy young males. *Clinical Hemorheology and Microcirculation* 47, 2, 121-129.
- [64] Monnier, JF. Benhaddad, AA. Micallef, JP. Mercier, J. Brun, JF., 2000. Relationships between blood viscosity and insulin-like growth factor I status in athletes. *Clinical Hemorheology and Microcirculation* 22,4, 277-286.
- [65] Peyreigne, C. Fédou, C. Benhaddad, AA. de Boisvilliers, F. Mercier, J. Bringer, J. Brun, JF., 1999. Hemorheology of growth hormone-deficient adults. *Clinical Hemorheology and Microcirculation* 20, 3, 167-174.
- [66] Benhaddad, AA. Monnier, JF. Fédou, C. Micallef, JP. Brun, JF., 2002. Insulin-like growth factor-binding protein 1 and blood rheology in athletes. *Clinical Hemorheology and Microcirculation* 26, 3, 209-217.
- [67] Mahran, A. Abdelmeged, A. Shawki, H. Moheyelden, A. Ahmed, AM., 2016. Nitric oxide donors improve the ovulation and pregnancy rates in anovulatory women with polycystic ovary syndrome treated with clomiphene citrate: A RCT. *International Journal of Reproductive Biomedicine* 14, 1, 9-14.
- [68] Gutarra-Vilchez, RB. Bonfill Cosp, X. Glujovsky, D. Viteri-García, A. Runzer-Colmenares, FM. Martinez-Zapata, MJ., 2018. Vasodilators for women undergoing fertility treatment. *Cochrane Database of Systematic Reviews* 10, 10, CD010001.
- [69] Duffy, DM. Ko, C. Jo, M. Brannstrom, M. Curry, TE., 2019. Ovulation: parallels with inflammatory processes. *Endocrine Reviews* 40, 2, 369-416.
- [70] Zackrisson, U. Löfman, CO. Janson, PO. Wallin, A. Mikuni, M. Brännström, M., 2011. Alterations of follicular microcirculation and apex structure during ovulation in the rat. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 157, 2, 169-174.
- [71] Mitsube, K. Brännström, M. Haraldsson, B., 2012. Modulation of microvascular permeability in the preovulatory rat ovary by an ovulatory gonadotropin stimulus. *Fertility and Sterility* 99, 3, 903-909.
- [72] Dhabhar, FS., 2018. The short-term stress response – Mother nature’s mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Frontiers Neuroendocrinology* 49, 175-192.
- [73] Ehrly AM. Landgraf, H. Hessler, J. Saeger-Lorenz, K., 1988. Influence of videofilm-induced emotional stress on the flow properties of blood. *Angiology* 39, 4, 341-344.
- [74] Sakai, A. Nakano, H. Ohira, T. Hosoya, M. Yasumura, S. Ohtsuru, A. Satoh, H. Kawasaki, Y. Suzuki, H. Takahashi, A. Sugiura, Y. Shishido, H. Hayashi, Y. Takahashi, H. Kobashi, G. Ozasa, K. Hashimoto, S. Ohto, H. Abe, M. Fukushima health Management Survey Group., 2017. Persistent prevalence of polycythemia among evacuees 4 years after the Great East Japan Earthquake: A follow-up study. *Preventive Medicine Report* 5, 251-256.
- [75] Kario, K. Matsuo, T., 1995. Increased incidence of cardiovascular attacks in the epicenter just after the Hanshin-Awaji earthquake. *Thrombosis and Haemostasis* 74, 1207.
- [76] Austin, AW. Patterson, SM. von Känel, R., 2011. Hemoconcentration and hemostasis during acute stress: interacting and independent effects. *Annals of Behavioral Medicine* 42, 2, 153–173.
- [77] Pfafferott, C. Zaninelli, R. Bauersachs, R. Volger, E., 1987. In vitro effect of norepinephrine and isoprenaline on erythrocyte filterability under pathologic conditions. *Clinical Hemorheology and Microcirculation* 7, 409 (abstract).
- [78] Hilario, S. Saldanha, C. Martins-Silva, J., 1999. The effect of adrenaline upon human erythrocyte. Sex-related differences? *Biorheology* 36, 124 (abstract).
- [79] Semenov, AN. Shirshin, EA. Muravyov, AV. Priezzhev, AV., 2019. The effects of different signaling pathways in adenylyl cyclase stimulation on red blood cells deformability. *Frontiers in Physiology* 10, 923.
- [80] Tikhomirova, IA. Muravyov, AV. Kruglova, EV., 2008. Hormonal control mechanisms of the rheological properties of erythrocytes under physical exercises and stress. *Biorheology* 45, 1-2, 49 (abstract).
- [81] Dhabhar, FS. McEwen, BS., 2007. Bidirectional effects of stress on immune function: possible explanations for salubrious as well as harmful effects. In: Ader, R. editor. *Psychoneuroimmunology IV*. San Diego: Elsevier. p. 723–760.