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Dear Dr. Nemeth,

your revised manuscript

Zoltan Klarik, Ferenc Kiss, Iren Miko and Norbert Nemeth:
Aorto-porto-caval micro-rheological differences of red blood cells in
laboratory rats: further deformability and ektacytometrial osmoscan data

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Sincerely

F. Jung
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Aorto-porto-caval micro-rheological differences of red blood cells in laboratory rats: further deformability and ektacytometrial osmoscan data

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Abstract

Investigation of regional hemorheological properties are important in ischemia-reperfusion experimental surgical and microsurgical research models, however, the physiological arterio-venous as well as porto-caval differences in erythrocyte deformability and aggregation are still controversial. In this study, besides measuring blood pH, blood gases, lactate concentration and hematological parameters we determined erythrocyte deformability (rotational ektacytometer) together with osmoscan data as well as erythrocyte aggregation (light transmittance aggregometer) in blood samples taken from the portal vein, caudal caval vein and abdominal aorta of rats. Blood pH, partial blood gas pressures showed the anticipated physiological differences. Leukocyte count was lower in arterial blood; hematocrit, erythrocyte and platelet count was higher in systemic and portal venous blood. The lowest elongation index values were measured in arterial blood, the highest in venous and portal venous blood showed values in between. The osmoscan data did not show important differences. Erythrocyte aggregation M index 5s showed the lowest values in arterial, higher in systemic venous and portal venous blood samples. M index 10s showed significantly low values in systemic venous blood. M1 values were slightly higher in arterial blood compared to venous blood but the highest were in portal venous blood. Erythrocyte deformability and aggregation may show aorto-porto-caval differences in the rat. The appropriate control examinations thus are important in experimental surgical and microsurgical research models.

Keywords: hemorheology, porto-caval difference, arterio-venous difference, red blood cell deformability, osmoscan, rat

1. Introduction

In the last decade, by using modern hemorheological devices, increasing amount of data became available on regional hemorheological properties as well as local versus systemic changes of red blood cell deformability and red blood cell aggregation in various pathophysiological conditions, including circulatory disorders, ischemia-reperfusion and vascular anastomoses [e.g. 7, 10, 14, 15, 18, 19, 25, 26]. The small volume of required blood sample for these laboratory tests (microliter-amount) and the sophisticated methodology provided the opportunity for further investigation of arterio-venosus micro-rheological differences in laboratory animals [13, 14, 29], or as laboratory standardization concerns, the systemic venous versus capillary blood samples in human [28]. The standardization of blood sampling methods and sites are important for the experimental surgical and microsurgical research models, too.

However, the data are still controversial. In a previous rat study we found higher aggregation index values (M1) with lower elongation index values in arterial (abdominal aorta) compared to venous (caudal caval vein) blood samples [13]. These kind of arterio-venosus hemorheological differences have been also demonstrated independently by other groups [29]. Other studies did not show important arterio-venous differences [15], and there are data on higher aggregation index values in venous blood [18, 20, 29]. Furthermore, several experimental models suggested that besides arterio-venous and local versus systemic alterations [6, 7, 19, 25], marked porto-caval hemorheological differences [7, 10] have to be taken under consideration when planning experiments and evaluating results. In the literature, we could not found any valuable and organized data about porto-caval hemorheological base differences.

Optimal osmotic environment, membrane stability and osmotic resistance are important parameters of red blood cells [9]. Any changes of red blood cell volume -because of osmotic micro-environmental alterations- may influence the deformability. Therefore, for

better understanding the *in vivo* hemorheological profile, comparative studies in this issue are still needed [5]. For analyzing such micro-rheological -supposedly minor- differences, sensitive and modern devices are needed. Son et al. [29] as well as Hever et al. [13] when investigating arterio-venous hemorheological differences in rats, used slit-flow ektacytometer and light transmittance aggregometer [1, 12].

Although the sensitivity of the available ektacytometers are relatively close to each other and their results are partially compatible [2], we aimed to investigate this topic further and to give novel data on arterial, venous and portal venous hemorheological base differences in rats, using the latest rotational ektacytometer together with the promising osmoscan function.

2. Methods

2.1. Experimental animals and blood sampling

The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 37/2007. and 6/2008. UD CAR), in accordance with the relevant Hungarian Animal Protection Act (Law XXVIII/1998) and EU Directives (EEC 63/2010).

Thirteen healthy male (n=8; 381.5 ± 13.4 g) and female (n=5; 292.2 ± 20.9 g) Sprague-Dawley rats (Janvier Co., France) were subjected to the study. Under general anesthesia (60 mg/kg, i.p., Thiopenthal®, Biocheme GmbH, Austria) midline laparotomy was performed and the infrarenal part of the abdominal aorta and the caudal caval vein as well as the portal vein were gently prepared and isolated using atraumatic, microsurgical methods.

Blood samplings were carried out by 26 G needle connected to a syringe (anticoagulant: Na-EDTA, 1.5 mg/ml). The vessels were punctured in the following order:

portal vein, caudal caval vein, abdominal aorta. 0.6-0.8 ml blood was withdrawn per vessel. At the end of the experiment the animals have been sacrificed by exsanguination.

The samples were immediately taken into the laboratory to complete the measurements within the possibly shortest time (total *in vitro* time was < 30 min).

2.2. Laboratory tests

2.2.1. Lactate level, blood pH and gas analysis

A blood gas analyzer automate (ABL555 Radiometer Copenhagen, Denmark) was used to determine lactate concentration (mmol/l), blood pH as well as pCO_2 and pO_2 values [mmHg]. The samples, in closed system, were immediately filled into the device without direct contact with air.

2.2.2. Hematological parameters

A semi-automated microcell counter (Sysmex F-800, TOA Medical Electronics Co., Japan) was used to determine the general hematological parameters.

2.2.3. Red blood cell deformability

A LoRRca MaxSis Osmoscan device (Mechatronics BV, The Netherlands) was used to measure red blood cell elongation index in the function of shear stress, together with red cell osmotic properties.

Red blood cell deformability measurements: trying to avoid direct contact with the laboratory air 5 µl of blood was taken into 1 ml of isotonic polyvinyl-pyrrolidone solution (360 kDa PVP in normal phosphate buffered saline; viscosity = 28.8 mPa.s, osmolarity = 305 mOsm/kg ; pH = 7.36) and gently mixed. The suspension was injected into the bob-cup system of the device, without air-bubbles. The LoRRca generated shear stress (SS) range from

0.3 to 30 Pa in computer-controlled grades, while the laser diffraction pattern was continuously analyzed: elongation index (EI) is equal to (L-W)/(L+W), where L is the length and W is the width of the diffractogram (reflecting deformed cells) at a constant shear stress [1, 12]. EI increases with red blood cell deformability. The measurements were carried out at constant temperature of 37 °C.

For comparison of individual EI-SS curves Lineweaver-Burk analyses were performed, calculating the maximal elongation index (EI_{max}) and the shear stress at half EI_{max} ($SS_{1/2}$ [Pa]) values, according to the following formula: $1/EI = SS_{1/2}/ EI_{max} \times 1/SS + 1/EI_{max}$

$SS_{1/2}$ increases with decreasing red blood cell deformability [3]. For comparison we used self-made calculation (shear stress range: shear stress range: 0.95-30 Pa) and the LoRRca software-given data, too (shear stress range: ~1.69-30 Pa).

Osmoscan function: 250 µl blood was taken into 5 ml PVP solution then gently mixed. The device generated a continuous shear stress of 30 Pa on the sample, while continuously measuring the elongation index. During the measurements the device changes the osmolarity of the medium using gradual mixtures of PVP solutions of 0 and 500 mOsmol/kg.

The measured and calculated parameters by the device: maximal elongation index values measured at shear stress of 30 Pa (maximal EI), minimal elongation index values measured at shear stress of 30 Pa and at low osmolar environment (Minimal EI), measureable elongation index values at shear stress of 30 Pa and high osmolar environment (EI_{hyper}), osmolarity at minimal EI, osmolarity at maximal EI (‘optimal‘ osmolarity), osmolarity at EI_{hyper} and the area under the individual osmolarity-elongation index curves (Figure 1).

2.2.4. Red blood cell aggregation

A Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used to measure red blood cell aggregation. The technique is based on light transmittance method.

Since the LoRRca device needs approximately 1 ml of blood for aggregation measurement, we could not use that function in this experiment. The Myrenne aggregometer requires approximately 20 µl of blood, only.

After disaggregating blood sample by 600 s^{-1}, the shear rate drops to zero (M index) or to a low, 3 s^{-1} shear rate (M1 index). According to the changes in light transmittance (disaggregation: low light transmittance, aggregation process: increasing light transmittance), the instrument calculates the aggregation index values at the 5^{th} or 10^{th} second of the aggregation process [12]. The indices (M 5 s, M1 5s, M 10 s, M1 10 s) increase with enhanced red blood cell aggregation.

2.3. Statistical analyses

For comparison of arterial, venous or portal venous blood samples paired t-test/Wilcoxon signed rank test as well as t-test/Mann-Whitney rank sum test were carried out according to the data distribution normality. A p value of <0.05 was considered as statistically significant.

3. Results

3.1. Lactate concentration, blood pH and gas values

Lactate concentration was slightly but significantly higher in arterial blood samples (p=0.001 by t-test and p=0.007 by paired t-test vs. venous sample; and only p=0.064 by t-test and p=0.073 by paired t-test vs. portal venous sample). *Blood pH* was also higher in arterial blood (p=0.002 by t-test and p<0.001 by paired t-test vs. venous sample; and p=0.008 using t-test and p=0.006 by paired t-test vs. portal venous sample), while in systemic venous and portal venous samples the values were almost identical (Table 1).

Blood gas values reflected the physiological expectations, pO_2 was the highest in arterial blood samples (p<0.001 both by t-test and paired t-test vs. venous sample; p<0.001 both by t-test and paired t-test vs. portal venous sample) and pCO_2 in systemic venous samples (p=0.028 using t-test and p=0.016 by Wilcoxon test vs. venous sample; and only p=0.063 by t-test and p=0.082 using paired t-test vs. portal venous sample) (Table 1).

3.2. Hematological parameters

Total *leukocyte count* was significantly lower in arterial blood compared to both systemic venous (p=0.013 by Mann-Whitney test and p<0.001 by paired t-test) and portal venous blood (p=0.009 using t-test and p=0.002 by paired t-test) (Table 2).

Although neither one reached the significant level, both *red blood cell count* and *hematocrit* were moderately elevated in systemic venous and portal venous blood samples. *Mean corpuscular volume* did not differ markedly, however, in venous samples slightly decreased.

Platelet count moderately elevated in systemic venous and portal venous samples without significant difference. The other tested hematological parameters did not differ essentially (Table 2).

3.3. Red blood cell deformability

Figure 2 shows elongation index (EI) – shear stress (SS [Pa]) peak centered cumulated curves of arterial, systemic venous and portal venous blood samples. Although the standard deviations overlapped, the differences were clearly observable between EI-SS curves: the highest EI values could be measured in arterial, the lowest values in systemic venous blood samples, while the portal venous values lay in between.

Table 3 presents the comparative data of EI-SS curves. *EI values at shear stress of 3 Pa* of arterial blood samples were significantly lower compared to systemic venous (p=0.036 by t-test and p=0.014 by paired t-test) and portal venous blood samples (p=0.039 by t-test but not significant when using paired t-test).

The parameterizational data also reflected these differences. EI_{max} values were significantly lower in arterial samples compared to systemic venous blood (p=0.004 by Mann-Whitney test and p=0.003 using Wilcoxon test; software-calculated values: p=0.003 by Mann-Whitney test and p=0.005 using Wilcoxon test).

$SS_{1/2}$ values were higher in arterial blood compared to systemic venous blood (p=0.023 by paired t-test; software-calculated values: p=0.043 by paired t-test). The difference between arterial and portal venous blood did not reach the statistically significant level (p=0.071 by paired t-test) (Table 3).

Figure 3 shows representative, peak-centered cumulated EI-osmolarity curves. Table 4 presents numerical data of all the osmoscan measurements in arterial, systemic venous and portal venous blood samples. In portal venous samples often slightly distorted curves could be seen with fluctuating values and flattening of the curve at higher osmolarity values (over 350-400 mOsm/kg).

The *minimal EI values* (at the lowest osmolarity where swelled red blood cells are still existing) were slightly higher in systemic venous and portal venous blood samples compared to the arterial blood. *Maximal EI* values did not differ essentially, however, in venous blood samples it was moderately higher. The EI_{hyper} (at the highest osmolarity where shrunk erythrocytes are still existing) were moderately higher in both venous sample-types compared to arterial blood.

The *osmolarity at the maximal EI ('optimal' osmolarity)* was a bit higher in systemic venous blood compared to arterial samples. In portal venous blood these values were the

lowest, almost reaching the significance level versus systemic venous vales (p=0.072 by paired t-test) (Table 4).

3.4. Red blood cell aggregation

Figure 4 shows M (shear rate: 0 s^{-1}) and M1 index values (shear rate: 3 s^{-1}) measured at the 5th or at the 10th second of the aggregation process.

In arterial blood M values at the 5th second showed the lowest values (1.15 ± 0.46) compared to caudal caval venous (1.26 ± 0.52) and portal venous blood samples (1.21 ± 0.58) but without significant difference (Figure 4A).

M1 values at the 5th second were higher in arterial blood samples (2.03 ± 0.88) compared to the venous blood (1.87 ± 0.85). In portal venous blood we measured the highest values (2.95 ± 2.03; p=0.055 vs. venous blood, using t-test) (Figure 4B).

M index at the 10th second showed marked differences. Caudal caval vein blood samples had the lowest values (2.27 ± 0.83), being significant compared to both arterial (4.38 ± 2.51; p=0.001 by Mann-Whitney test) and portal venous blood samples (4.71 ± 1.98; p<0.001 by using Mann-Whitney test) (Figure 4C).

M1 index at the 10th second did not show significant differences. However, arterial blood samples had slightly higher values (5.27 ± 3.13) compared to the venous blood (5.03 ± 2.24), while portal venous samples showed the highest values (6.18 ± 4.24) (Figure 4D).

4. Discussion

In man, the portal vein carries 70-75% of the total blood flow to the liver, by about 1.0-1.2 ml/min flow rate [17]. The portal venous blood is definitely postcapillary type, being partly deoxygenated. Blood pH and blood oxygenation-level are known to affect red blood cell deformability and red blood cell aggregation [4, 8, 16, 24, 31]. Unfortunately, only a few

number of studies are dealing with the changes of red blood cell deformability and red blood cell aggregation being investigated in parallel with acid-base, blood gas parameters or blood pH. The studies often show controversial data, and the magnitude and reversibility of the micro-rheological changes are still unclear [21, 24].

Furthermore, differences in hematocrit and fibrinogen concentration at various sites of the circulation may also have a slight impact of red blood cell aggregation [4, 11, 19, 28]. However, there is no correct and uniform explanation for the arterio-venous or arterio-capillary-venous and porto-caval differences.

The majority of data referring to arterio-venous or porto-caval micro-rheological differences are originated from experimental studies, which deal with ischemia-reperfusion of limbs [15, 26, 30], small intestine [7], liver [10], cerebral hypo- and hyperperfusion [18, 25] and artificial arterio-venous shunt models [14]. However, there is a lack of targeted comparative hemorheological analysis for exploring these arterio-venous or porto-caval micro-rheological relations. With the modern hemorheological devices -requiring small blood sample volume- still little number of studies provide comparative data [28, 29].

In this current study we aimed to analyze the possible aorto-porto-caval differences of red blood cell deformability (together with osmoscan data), red blood cell aggregation, hematological parameters as well as blood pH, lactate concentration, pCO_2 and pO_2 values.

As anticipated physiologically, the highest pO_2 values were measured in arterial blood, lower values in portal and systemic venous blood samples. The lowest pCO_2 values were found in arterial blood, the highest in venous blood and the portal blood in between. Blood pH was the lowest in systemic and portal venous blood, however, the lactate concentration was also lower compared to arterial blood.

Investigating the hematological parameters we found that total leukocyte count was lower in arterial blood compared both to systemic and portal venous blood. We found similar

arterio-venous relation (lower WBC count in arterial blood) in previous studies [13, 30]. It is supposed that the difference might be originated from the relative distributional differences of leukocytes or by the order of the blood samplings (the last sampling site was the abdominal aorta). However, red blood cell count (calculated from the measured hematocrit and MCV values by the microcell counter), hematocrit and platelet count were higher in systemic venous and portal venous blood.

Concerning the red blood cell deformability data, the differences among arterial, systemic and portal venous blood were well visible in this experiment. Interestingly, the lowest elongation index values have been measured in arterial blood, the highest in systemic venous blood and the portal venous blood showed EI values in between. The parametrizational data of EI-SS curves also reflected these differences. Herein we compared the EI_{max} and $SS_{1/2}$ values in two shear-stress ranges: 0.95-30 Pa (self-made calculation [3]) and ~ 1.69-30 Pa (automatic calculation by the LoRRca software). Both calculations showed similar magnitude of the differences, except for $SS_{1/2}$ values in blood samples taken from the caudal caval and portal veins.

Interestingly, in female animals the aorto-porto-caval differences were observable at a smaller degree (data was not analyzed separately by statistics). However, it has been demonstrated, that female rats have higher elongation index values compared to males [23].

Son et al. also found that EI values were lower in arterial blood compared to venous blood in rats, however, this difference could not be observed in human or in canine [29]. The arterio-venous red blood cell deformability difference and its alteration could be also detected in an artificial arterio-venous shunt model in the rats [14]. In that model end-to-side shunts have been performed between the saphenous artery and the medial saphenous vein on the left hind limb of rats, using microsurgical techniques. The right side served as control. The slight

arterio-venous differences that could be measured in the samples taken from the control side saphenous vessels, disappeared at the shunt side, showing lower EI values [14].

For data comparison, we have used two ways for statistical analysis. The first was a simple inter-group comparison with t-test or Wilcoxon signed rank test (depending on the normality of data distribution), if we considered arterial, venous and portal venous blood sample-types as three different kind of sample types to be evaluated. The other way was the application of paired t-test or Wilcoxon test (again depending on the normality of data distribution), since the same 'blood organ' was sampled but at various sites. Thus, a kind of before-after comparison was also performed, like evaluating and roughly comparing the blood micro-rheological state before entering the capillary-territories of the intestine (abdominal aorta versus portal vein), liver (portal vein versus caudal caval vein) and other tissues in general (abdominal aorta versus caudal caval vein). In the majority of cases, when we found significant differences, both ways of comparison resulted in a p value less than 0.05. Of course, the rheological differences must be more sophisticated at various levels of the circulation, and the *in vivo* conditions are very complex [5, 6, 27]. However, for sampling we had to choose large vessels to be punctured and letting enough blood volume for all the laboratory tests.

The osmoscan data did not show important aorto-porto-caval differences. However, it could be observed that samples from the portal veins often showed more expressed fluctuating values and moderately flattened plot over 350-400 mOsm/kg. Since very little data are available with the new osmoscan, we could not find explanation for these results.

The red blood cell aggregation data showed more obvious differences. The four variables (M and M1 index values at 5 s and at 10 s) did not show the same differences. M index at 5 s showed the lowest values in arterial, higher in systemic venous and portal venous blood samples. M index at 10 s showed the (significantly) lowest values in systemic venous

blood. When testing M1 values, the arterial blood samples showed slightly higher values compared to the caudal caval venous blood and the highest values in portal venous blood.

In an earlier study we found that arterial blood taken from the abdominal aorta showed higher aggregation index M1 values (at shear rate of 3 s^{-1}) in rats, compared to venous blood sampled from the caudal caval vein [13]. In that study, M values (at stasis) could not be tested in all the samples, many tests resulted zero values, thus, convincing comparison and statistical analyses could not be performed. In this current study, we could obtain enough data for a better comparison. There were only 3 cases, when the aggregation index values showed 0.0 values. These samples were excluded from the data comparison. Interestingly, the current results also demonstrated that M1 index (both at the 5^{th} and 10^{th} seconds) may show higher values in arterial blood samples compared to the caval venous blood. However, M index at the 5^{th} second represented the lowest values in arterial blood. In contrast, M index at the 10^{th} second showed obvious differences.

According to our experiences, the red blood cell aggregation measurements by light transmittance method in rat blood often have difficulties (e.g. zero values, M>M1 values), therefore it is recommended to use appropriate control groups in all experiments. It is also recommended to perform the measurements very soon after sampling because it has been demonstrated that the aggregation index values of rat may decrease rapidly and significantly during *in vitro* storage, resulting in misleading data [22].

5. Conclusion

Micro-rheological variables, such as red blood cell deformability and red blood cell aggregation may show arterio-venous and porto-caval differences in the rat. The appropriate control examinations thus are important in experimental surgical and microsurgical research

models. For better understanding the significance and the background of these differences, further comparative studies are needed.

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8. Tables

Table 1. Lactate concentration, blood pH values and partial blood gas pressure data of samples taken from the abdominal aorta, caudal caval vein and portal vein.

Variable	Abdominal aorta	Caudal caval vein	Portal vein
lactate [mmol/l]	2 ± 0.42 *	1.3 ± 0.24	1.57 ± 0.39
pH	7.39 ± 0.03 * #	7.33 ± 0.03	7.32 ± 0.05
pO_2 [mmHg]	87.7 ± 10.32 * #	41.5 ± 10.86	39.5 ± 7.98
pCO_2 [mmHg]	33.01 ± 7.63 *	44.67 ± 10.87	42.26 ± 10.45

means ± S.D.

* p<0.05 vs. caudal caval vein, # vs. portal vein

Table 2. Hematological parameters in blood samples taken from the abdominal aorta, caudal caval vein and portal vein.

Variable	Abdominal aorta	Caudal caval vein	Portal vein
WBC count [$x10^3$/µl]	4.43 ± 1.54 * #	5.94 ± 1.67	5.86 ± 1.34
RBC count [$x10^6$/µl]	6.98 ± 0.42	7.21 ± 0.4	7.23 ± 0.39
Hgb [g/dl]	11.71 ± 0.58	12.06 ± 0.52	12.04 ± 0.63
Hct [%]	44.46 ± 2.2	45.38 ± 2.15	44.74 ± 1.96
MCV [fl]	63.76 ± 2.27	62.99 ± 1.73	61.91 ± 2.04
RDW-CV% [%]	13.83 ± 0.41	13.78 ± 0.52	13.85 ± 0.45
MCH [pg]	16.78 ± 0.59	16.75 ± 0.43	16.66 ± 0.55
MCHC [g/dl]	26.36 ± 0.66	26.59 ± 0.39	26.92 ± 0.75
Plt count [$x10^3$/µl]	735.2 ± 46.5	742.4 ± 76.1	747.8 ± 75.1
MPV [fl]	7.79 ± 0.41	7.61± 0.37	7.54 ± 0.37

means ± S.D.

* p<0.05 vs. caudal caval vein, # vs. portal vein

Table 3. Comparative parameters of elongation index (EI) – shear stress (SS [Pa]) curves, describing red blood cell deformability in blood samples taken from the abdominal aorta, caudal caval vein and portal vein.

Variable	Abdominal aorta	Caudal caval vein	Portal vein
EI at 3 Pa	0.331 ± 0.034 *#	0.351 ± 0.014	0.346 ± 0.032
EI_{max} A	0.546 ± 0.038 *	0.575 ± 0.022	0.557 ± 0.043
EI_{max} B	0.529 ± 0.039 *	0.558 ± 0.023	0.541 ± 0.039
$SS_{1/2}$ A [Pa]	2.07 ± 0.37 *	1.95 ± 0.31	1.93 ± 0.23
$SS_{1/2}$ B [Pa]	1.7 ± 0.18 *	1.59 ± 0.18	1.65 ± 0.15

means ± S.D.

EI at 3 Pa = elongation index at shear stress of 3 Pa; EI_{max} = calculated maximal elongation index; $SS_{1/2}$ = shear stress values at half maximal elongation index

A: self-made calculation using Lineweaver-Burk analysis [3] (shear stress range: 0.95-30 Pa)

B: automatic calculation by the LoRRca software (shear stress range: ~ 1.69-30 Pa)

* p<0.05 vs. caudal caval vein, # vs. portal vein

Table 4. Parameters of osmoscan measurements (at shear stress of 30 Pa) in blood samples taken from the abdominal aorta, caudal caval vein and portal vein.

Variable	Abdominal aorta	Caudal caval vein	Portal vein
Minimal EI	0.091 ± 0.015	0.097 ± 0.013	0.1 ± 0.015
Maximal EI	0.456 ± 0.06	0.466 ± 0.041	0.452 ± 0.075
EI_{hyper}	0.228 ± 0.03	0.233 ± 0.021	0.236 ± 0.025
Osmolarity at minimal EI [mOsm/kg]	166.5 ± 11.41	168.87 ± 13.26	166.71 ± 10.4
Osmolarity at maximal EI [mOsm/kg]	335.62 ± 33.51	345.25 ± 34.42	327 ± 23.21
Osmolarity at EI_{hyper} [mOsm/kg]	487.12 ± 28.66	489 ± 24.6	488.25 ± 25.01
Area (osm-EI curve)	117.63 ± 18.89	121.12 ± 13.61	117.91 ± 20.08

means ± S.D.

Maximal EI = maximal elongation index values measured at shear stress of 30 Pa

Minimal EI = minimal elongation index values measured at shear stress of 30 Pa, at low osmolar environment

EI_{hyper} = measureable elongation index values at shear stress of 30 Pa and high osmolar environment

9. Figure legends

Figure 1.

A characteristic curve of the elongation index (EI) - osmolarity values [mOsmol/kg] obtained from the osmoscan test, showing the comparative values (minimal EI, maximal EI, EI_{hyper}, osmolarity values at minimal EI, at maximal EI and at EI_{hyper}).

Figure 2.

Elongation index (EI) values in the function of shear stress (SS [Pa]) in blood samples taken from the abdominal aorta, caudal caval vein and portal vein.

means ± S.D.

Figure 3.

Representative elongation index (EI) - osmolarity curves [mOsmol/kg] in blood samples taken from the abdominal aorta, caudal caval vein and portal vein.

Figure 4.

Red blood cell aggregation index values in blood samples taken from the abdominal aorta, caudal caval vein and portal vein: M index values (shear rate: 0 s^{-1}) at the 5^{th} or at the 10^{th} second (**A**, **B**) and M1 index values (shear rate: 3 s^{-1}) measured at the 5^{th} or at the 10^{th} second (**C**, **D**) of the aggregation process.

means ± S.D., * $p<0.05$ vs. caudal caval vein, # vs. portal vein

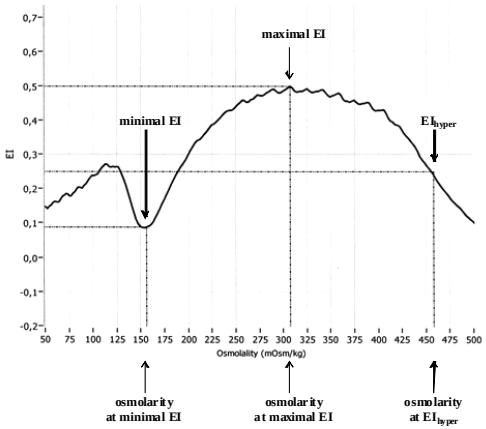

Figure 1.

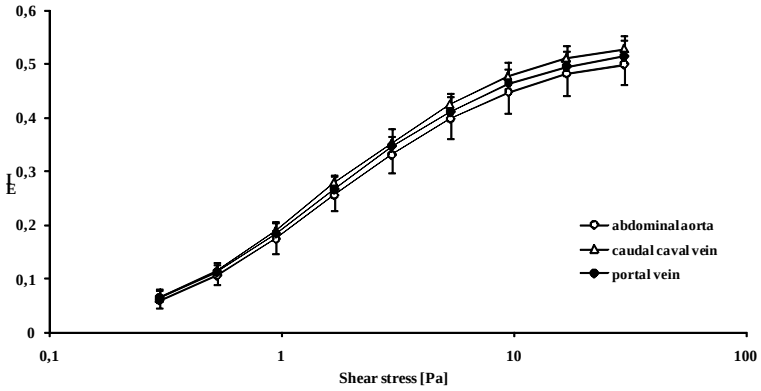

Figure 2.

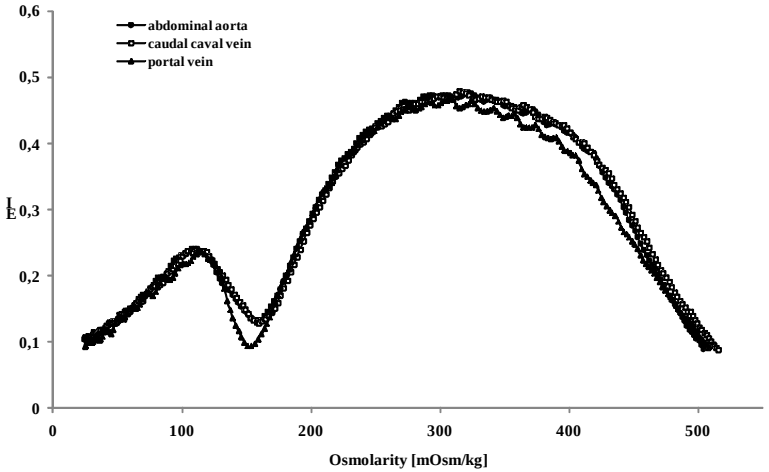

Figure 3.

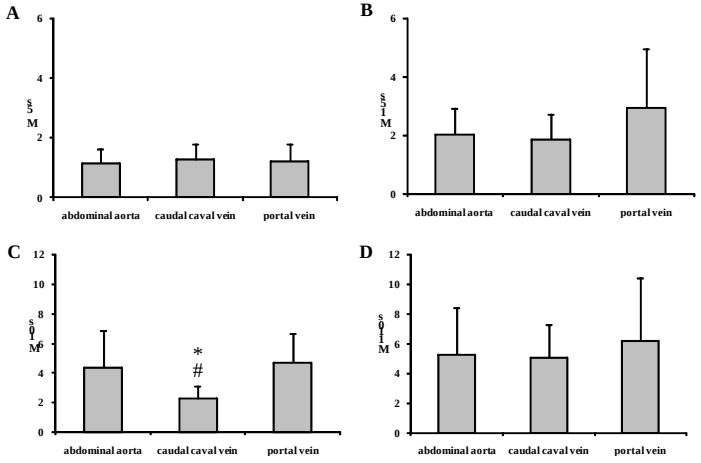

Figure 4.