

# Neuraxial anesthesia is less harmful to the endothelial glycocalyx during elective joint surgery compared to general anesthesia<sup>1,2</sup>

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## Abstract.

**BACKGROUND:** Damage of the endothelial glycocalyx (EG) has been described during surgery, but the effect of different anesthesia techniques remains unknown. Perfused boundary region (PBR) evaluated by side-stream dark field (SDF) imaging of the sublingual microcirculation enables *in vivo* EG assessment. PBR values are inversely related to the EG thickness.

**OBJECTIVE:** The aim of the observational study was to evaluate the changes of PBR in patients undergoing elective joint surgery under general (GA) vs. neuraxial anesthesia (NA). Our hypothesis was that PBR will be lower in patients in NA.

**METHODS:** Sixty consecutive patients (ASA 1–3) undergoing elective total knee or hip replacement under GA or NA were included in this prospective observational cohort study. PBR in the sublingual microcirculation was recorded in each patient using SDF at two time points - before surgery and 2 hours after surgery.

**RESULTS:** Before surgery, there was no significant difference in baseline PBR between groups (NA: 1.95  $\mu\text{m}$  ( $\pm 0.24$ ); GA: 2.02  $\mu\text{m}$  ( $\pm 0.26$ ;  $p = 0.098$ ). Postoperatively (2 hours after surgery) PBR was significantly increased in both groups with respect to baseline values (NA: 2.09  $\mu\text{m}$  ( $\pm 0.19$ ), GA: 2.20  $\mu\text{m}$  ( $\pm 0.25$ );  $p < 0.001$ ). In the GA group, postoperative PBR values were significantly higher than in the NA group ( $p = 0.006$ ).

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**CONCLUSION:** Joint surgery led to significant increases of PBR. Patients in the GA group had significantly higher PBR values 2 hours after surgery compared to NA group. This might implicate that NA is associated with less EG damage than GA in elective hip/knee surgery.

Keywords: Endothelial glycocalyx, neuraxial anesthesia, joint surgery

## List of abbreviations

EG endothelial glycocalyx  
GA general anesthesia  
ICU intensive care unit  
NA neuraxial anesthesia  
PBR perfused boundary region  
RBC red blood cells.

## 1. Background

Endothelial glycocalyx (EG) is a gel-like sugar-based structure, lining the apical surface of endothelial cells [1, 2]. It has been considered as a major regulator of endothelial functions [3–5]. The key role of EG is to modulate extravascular fluid shifts and prevent a direct contact between endothelial cells and circulating inflammatory or pro-coagulant triggers [6]. Importantly, EG represents not only a passive barrier between intravascular compartment and the endothelial surface, but it plays an important role in the regulation of inflammatory processes, ischemia and reperfusion injury, and modulation of endothelial production of nitric oxide by mechanotransduction of fluid shear stress [7, 8]. The delicate nature of its carbohydrate-based structure predisposes EG to be fragile and to respond rapidly to noxious stimulation of various origins and to date, the role of EG in clinical medicine is being increasingly appreciated [3]. In addition the composition and dimensions of EG fluctuates even in a physiological condition mainly due to the shear stress from the flowing blood elements and it is continuously regenerated by the synthesis from the endothelial cells [9]. The current evidence suggests that under virtually all critical conditions (e.g. sepsis, trauma, hypoxia) a distortion of the structure and function of EG may occur which in turn can contribute to the distortion of the microcirculation, thrombosis [10] and the subsequent development of organ dysfunction and organ failure [11, 12].

Visualization of the EG in humans is extremely difficult, mainly because of its microscopic dimension and fragility. Until recently, direct *in vivo* visualization of EG in humans has been unsuccessful. The EG is partially accessible to flowing red blood cells at its luminal side, a portion called the Perfused Boundary Region (PBR). The EG forms a mechanical, protective barrier that limits radial motion of flowing red blood cells towards the endothelial surface. Damage to glycocalyx results in increased PBR, which can be measured in human sublingual microvasculature recordings obtained by one of the imaging techniques currently available, e.g. Sidestream Dark Field (SDF) or just recently introduced Incident Dark Field (IDF) imaging technology [13, 14].

Changes in EG related to anesthesia and perioperative medicine are lacking detailed exploration so far [15] and more information about the effect of different treatment strategies [16] may significantly affect the way we look at anesthesia and related interventions in terms of their possible impact on EG.

## 68 2. Objective

69 The aim of this study was to evaluate PBR value changes as *in vivo* marker of EG thickness in  
70 patients undergoing elective hip/knee surgery under general (GA) and neuraxial anesthesia (NA). Our  
71 primary hypothesis was that NA affects EG less than GA due to the omission of oxidative stress from  
72 general anesthetics.

## 73 3. Methods

### 74 3.1. Study design and setting

75 A prospective observational study that has been approved by a local ethical committee and reg-  
76 istered at ClinicalTrials.gov (<https://clinicaltrials.gov>, ClinicalTrials.gov Identifier: NCT02783443).  
77 The study was performed at the University Hospitals Hradec Kralove and Plzen (Czech Republic).

### 78 3.2. Participants

79 Adult patients scheduled for elective total knee/hip replacement under GA and NA (either epidural  
80 or spinal) were included in this prospective observational study conducted at two university hospi-  
81 tals. Patients were recruited consecutively during a pre-admission clinic visit from October 2015 to  
82 December 2016. Inclusion criteria were: age above 18 years, American Society of Anesthesiologists  
83 Physical Status (ASA) 1–3, no history of any oncological disease, and not an active smoker. Patients  
84 were investigated after obtaining informed consent. Patients were excluded in the case of surgery  
85 cancellation or refusal of the second recording of the microcirculation. After enrolment to the study  
86 a designated physician followed patients until hospital discharge. GA group of patients were pre-  
87 medicated with morphine 0.1 mg/kg (Morphine, BB Pharma, Prague, Czech Republic) intramuscular  
88 injection. Induction of anesthesia by propofol 2 mg/kg (Propofol, Fresenius Kabi, Bad Homburg, Ger-  
89 many), sufentanil 0.2 µg/kg (Sufentanil Torrex, Chiesi CZ, Prague, Czech Republic) and atracurium  
90 0.4 mg/kg (Tracrium, Aspen Pharma, Dublin, Ireland). The airway was secured by orotracheal intuba-  
91 tion. Anesthesia and analgesia was maintained by sevoflurane 0.7–1 MAC (Sevorane, AbbVie, Prague,  
92 Czech Republic) in a fresh gas mixture with air and 0.4 F<sub>i</sub>O<sub>2</sub> and sufentanil 0.1 µg/kg. Balanced crys-  
93 talloid solution was used for fluid therapy (Ringerfundin, BBraun, Melsungen, Germany) at basal  
94 rate 2 ml/kg/hour with additional boluses as required during surgery according to the blood loss. NA  
95 group of patients were premedicated with oral bromazepam 1.5 mg (Lexaurin, Kabu Pharma, Prague,  
96 Czech Republic). Subarachnoid anesthesia was done by lumbar puncture (Quinke needle, 25 G, BBraun,  
97 Melsungen, Germany) and injection of 2ml of 0.5% levobupivacaine (Chirocaine, AbbVie, Prague,  
98 Czech Republic) and 2.5 µg of sufentanil intrathecally. Balanced crystalloid solution was used for  
99 fluid therapy (Ringerfundin, BBraun, Melsungen, Germany) at basal rate 2 ml/kg/hour with additional  
100 boluses as required during surgery according to the blood loss and blood pressure drop after subarach-  
101 noid blockade. Intraoperative sedation was induced and maintained by midazolam 1 mg intravenously  
102 (Accord Healthcare, North Harrow, UK) and sufentanil 5 µg intravenously. Supplementary oxygen  
103 was provided by face mask with flow of 5 L/minute. In both groups the blood pressure was kept in a  
104 range ± 20% from baseline. If vasopressor was required, an intravenous dose of 10mg of ephedrine  
105 (Ephedrin biotika, BB Pharma, Prague, Czech Republic) was used. The tourniquet was used only in  
106 knee replacement in patients in NA group on the thigh of the operated leg with pressure of 220 mmHg.  
107 The bone cement was used according to the type of alloplastic.

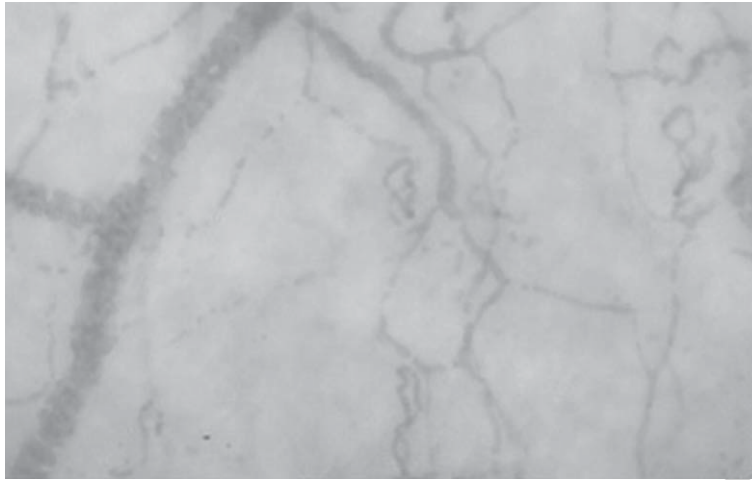


Fig. 1. The original image of the sublingual microcirculation. Original image of the sublingual microcirculation. Surface area: 915x686  $\mu\text{m}^2$ . Magnification: 325x. Recorded by SDF camera.

The primary outcome of the study was the change in PBR values 2 hours after surgery. Selected clinical and laboratory variables were also recorded preoperatively (age, gender, patient's history - any type of vascular disease, hypertensive on medication, diabetes mellitus (on insulin, oral antidiabetic agents or diet), concomitant medication - beta-blockers, statins, insulin, ACE inhibitors, calcium channel blockers, steroids or immunosuppressing drugs taken longer than one week prior the surgery, routinely ordered laboratory values), during and after anesthesia/surgery (heart rate, blood pressure, pulse oximetry, body temperature, type of anesthesia, type and length of surgery, major and minor intra-operative adverse events (a major adverse event was defined as a complication leading to unplanned admission to intensive care unit (ICU) within 48 hours, a minor adverse event was defined as a complication in the patient documentation that needed therapeutic intervention without admission to ICU), estimated blood loss, amount of fluids/colloids given, amount of transfusion units given during the surgery and up to 2 hours after the surgery.

### 3.3. Perfused boundary region

For each patient, we scheduled two successive visits at the bedside to obtain recordings of the capillary web of the sublingual microcirculation. The first visit was before surgery (T1) and the second 2 hours after surgery (T2). Patients were given a glass of lukewarm water to rinse the saliva off the sublingual mucosa. On each visit, we performed two recordings from one side of the sublingual area. Microcirculation videos were recorded by specialized hand-held video microscope (KK camera, Research Technology Limited, Alliance Court, Honiton, UK; Fig. 1) connected to a laptop computer with specialized recording and analysis software (GlycoCheck, Maastricht, the Netherlands).

Perfused boundary region (PBR) describes the extent of penetration of the flowing red blood cells (RBC) in  $\mu\text{m}$  into the luminal surface of the EG by measuring the radial motion of RBC away from the central flow towards the endothelial cells. The more the EG is injured, the deeper RBC penetrates into the glycocalyx and the higher the PBR is. The software automatically measures PBR in vessels of diameter ranging from 5 to 25  $\mu\text{m}$  (capillaries exclusively) and the resulting number stands for an average of PBR that is corrected for the potential changes in the distribution of vessel diameters. A detailed description of PBR calculation has already been described elsewhere [17]. Briefly, the software identifies all available vessels and places 10  $\mu\text{m}$  long vascular segments along them (Fig. 2).

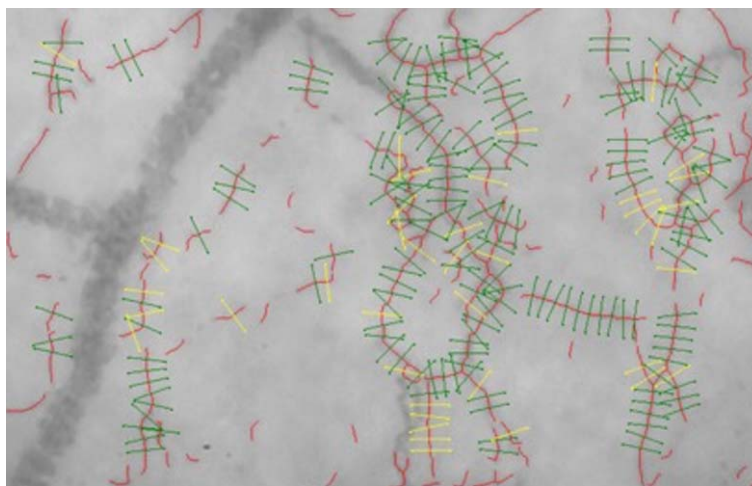


Fig. 2. Image of the software processing of the Fig. 1. Image processing by GlycoCheck analysis software. Red lines trace all available vessels with diameters from 5 to 25  $\mu\text{m}$ . Green lines are demarking vascular segments selected for the analysis. Yellow lines are bordering invalid segments.

The recording is stopped when 3000 segments are acquired in focus and without movement. Then the software selects segments with sufficient contrast with the background and counts the median RBC column width and its distribution from the intensity profile. From this intensity profile, the perfused diameter of the vessel is calculated by a linear regression analysis. The PBR stands for the distance between RBC column width and perfused diameter according to the equation:  $(\text{Perfused diameter} - \text{median RBC column width})/2$ .

### 3.4. Statistical analysis

Based on the expected difference in PBR between patients under GA and NA, the alpha error  $I = 0.05$  and study power = 0.99, sample size calculation was 52 patients. We aimed to enrol 60 consecutive patients to reach 30 patients with each type of anesthesia, either general anesthesia or neuraxial.

For statistical analysis, we used Graph Pad Prism v6.0c (GraphPad Software, Inc., CA, USA). All data were tested for normality prior testing by D'Agostino-Pearson omnibus normality test. Data are expressed as mean (and standard deviation) or median (and interquartile range), a value of  $p \leq 0.05$  was considered as statistically significant for paired and unpaired  $t$ -tests. Z-test we used for two populations proportion analysis.

## 4. Results

Sixty-three patients were initially enrolled; one patient was canceled before surgery due to a myocardial infarction and two patients refused to have their microcirculation be recorded again due to the irritating sensation in the mouth and nausea during the first recording. Thirty patients underwent hip/knee replacement surgery under general anesthesia (GA group) and thirty patients under neuraxial anesthesia (NA group). Baseline clinical characteristics of both groups are summarized in Table 1, except for bodyweight (significantly higher weight in GA group) there were no significant differences. There were also no significant differences in selected preoperative and postoperative variables - glycemia, natremia, creatinine and hemoglobin levels, and blood loss or fluid balance response. (Table 2).

Table 1  
Basic demographic and surgery data

<i>Patients' data</i>	GA	NA	<i>p value</i>
Age [years]	66.63 (7,08)	67.13 (9,81)	0.82
Gender [%]	F 53, M 47	F 47, M 53	0.19
Weight [kg]	91.46 (17,29)	81.30 (19,87)	0.04
Height [cm]	172.10 (8,42)	168.60 (10,92)	0.17
ASA			
I	2	0	0.07
II	21	19	0.54
III	7	11	0.13
<i>Type of surgery</i>			
Hip replacement	18	21	0.21
Knee replacement	12	9	0.21
Prime - implantation (hip or knee)	27	24	0.14
Revision surgery (hip or knee)	3	6	0.14

Baseline clinical characteristics of both groups. GA - general anesthesia, NA - neuraxial anesthesia. ASA - American Society of Anesthesiologists physical status. M - male, F - female. Data are presented as mean (standard deviation).

Table 2  
Selected laboratory and intraoperative course data

<i>Patients' data</i>	GA	NA	<i>p value</i>
Glycemia [mmol/l]	5.4 (5–6)	5.55 (4.88–6.43)	0.53
Natremia [mmol/l]	140 (2,42)	139.50 (2,35)	0.42
Creatinine [mmol/l]	73 (62.70–77.00)	77 (67.50–86.50)	0.17
Hemoglobin [g/l]	140.90 (13,72)	139.30 (15,97)	0.68
Hematocrit [%]	41.23 (3,72)	42.03 (4,46)	0.45
Blood loss [ml]	400 (237.50–600)	300 (300–500)	0.68
Fluid balance [ml]	1175 (909,80–1638)	1550 (975–1800)	0.22

Variables related to the intraoperative course. GA - general anesthesia, NA - neuraxial anesthesia. Data are presented as mean (standard deviation) or median (interquartile range).

161 Patients' medical history is summarised in Table 3. The tourniquet was used in knee replacement  
 162 surgery in patients in NA (9 patients) as well as bone cement. Before surgery, there was no significant  
 163 difference in PBR between groups (1.95  $\mu\text{m}$  ( $\pm 0.24$ ) in NA vs. 2.02  $\mu\text{m}$  ( $\pm 0.26$ ) in GA,  $p = 0.098$ ).  
 164 Two hours after surgery PBR significantly increased in both groups with respect to baseline values  
 165 (NA: 2.09  $\mu\text{m}$  ( $\pm 0.19$ ),  $p < 0.001$ ; GA: 2.20  $\mu\text{m}$  ( $\pm 0.25$ ),  $p < 0.001$ ). Moreover, PBR significantly  
 166 differed between NA and GA group 2 hours after surgery ( $p = 0.006$ ) (Table 4, Fig. 3). In NA group, 3  
 167 patients (10%) were reported to have a postoperative adverse event. In GA group 14 patients (47%),  
 168 the difference reached statistical significance ( $p < 0.001$ ). Major adverse events were a pulmonary  
 169 embolism (one patient in NA group), myocardial infarction (one patient in NA group), deep venous  
 170 thrombosis (one patient in GA group) and supraventricular arrhythmia (one patient in GA group).  
 171 Table 5 summarizes minor postoperative adverse events in both groups.

Table 3  
Summarisation of patients' medical history

Patients' history	GA	NA	<i>p</i> value
Hypertension	21	18	0.42
Antihypertensive drugs	21	18	0.42
Diabetes mellitus	5	9	0.22
PAD/insulin	3	7	0.16
Dyslipidaemia	9	10	0.78
Statins	9	10	0.78

GA – general anesthesia, NA – neuraxial anesthesia, PAD – per oral antidiabetic drugs.

Table 4  
The endothelial glycocalyx analysis data

PBR [ $\mu$ m]	GA	NA	<i>p</i> value
PBR	2.02 (0.26)	1.95 (0.24)	0.098
PBR_2	2.20 (0.25)	2.09 (0.19)	0.006
		PBR – PBR_2	<0.001
	PBR – PBR_2		<0.001

Results of the endothelial glycocalyx measurement. PBR - perfused boundary region, PBR\_2 – perfused boundary region in 2 hours after the surgery, GA - general anesthesia, NA - neuraxial anesthesia. Data are presented as mean (standard deviation).

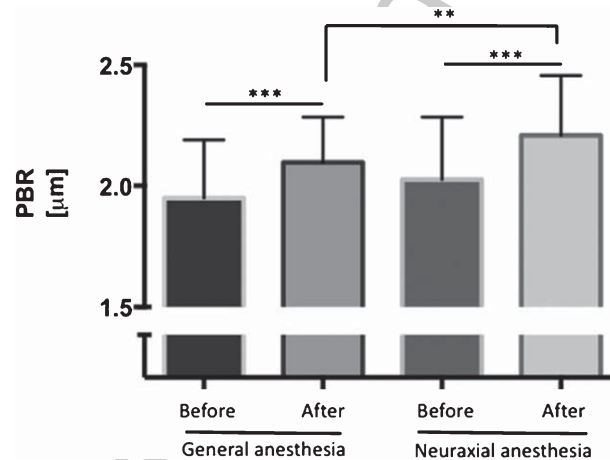


Fig. 3. Graph of the endothelial glycocalyx analysis data. PBR results in both groups. PBR - perfused boundary region. Data are presented as mean (columns) and standard deviation (error bars).

## 5. Discussion

Our study showed that elective joint surgery led to increased PBR dimensions and that patients in GA group had higher PBR 2 hours after surgery compared to NA group. We have chosen this interval of 2 hours after surgery because patients had entirely recovered from both types of anesthesia, and

Table 5  
Postoperative adverse events

<i>Minor postoperative adverse events</i>	GA	NA	<i>p value</i>
Anemia	2	0	0.07
Obstipation	0	1	0.15
PONV	8	0	0.001
Delirium	2	0	0.07
Total	12	1	<0.001

Summary of minor postoperative adverse events.  
PONV - postoperative nausea and vomiting.

176 in addition EG regenerates in days back to full thickness. Therefore, 2 hours after the surgery, the  
177 presumed damage would be best detectable [2]. Since PBR is an indicator of endothelial glycocalyx  
178 thickness, obtained data supports the hypothesis that neuraxial anesthesia techniques are associated  
179 with a lower extent of EG damage compared to general anesthesia for the given surgical procedure.  
180 Different types of surgery under general anesthesia have been associated with glycocalyx injury [18, 19]  
181 and a term “endotheliopathy” has been just recently introduced in order to describe changes of vascular  
182 endothelium with regard to trauma, which has many common features with elective surgery [20]. There  
183 is ongoing discussion whether or not the choice of the anesthetic may affect the outcome after total hip  
184 replacement [21–24] and there is limited evidence favouring neuraxial anesthesia techniques in term of  
185 better postoperative outcome as shown in a recent systematic review by Johnson et al. [24]. The vascular  
186 endothelium is one of the earliest sites of injury during inflammation [25, 26], ischemia-reperfusion  
187 injury [25, 27] and trauma [12, 15, 28] - all those events are present during surgery to some extent.  
188 Attenuating endothelial injury could be the result of better blocking afferent inputs from the surgical  
189 field, reduced systemic inflammatory response to surgery and more efficient sympathetic blockade  
190 attributed to neuraxial blockade [29]. However, further mechanisms may play a role - less amount of  
191 intravenous anaesthetics given, modulating vascular tone or modulating cell to cell interaction by local  
192 anaesthetic agents [29, 30].

193 Our study has several limitations. Firstly, we used PBR as the only method for the evaluation of EG  
194 damage. PBR describes the extent of penetration of the flowing RBC in  $\mu\text{m}$  into the EG by measuring  
195 the deviation of RBC from the central flow towards the endothelial cells. EG damage results in deeper  
196 penetration of RBC and is reflected by increased PBR value. Despite the fact that PBR was used  
197 as an indicator reflecting glycocalyx thickness in various clinical scenarios [9, 35–38], the current  
198 report of peri-operative glycocalyx changes would benefit from combining PBR with measuring other  
199 humoral markers (e.g. syndecan–1, hyaluronan, heparan sulfate) to estimate glycocalyx shedding *in*  
200 *vivo*. However, none of these circulating glycobiological markers are specific to endothelia. Moreover,  
201 they are strongly affected by extravascular tissue damage and changes in hepatic function which is a  
202 major obstacle to their use in clinical medicine [26, 35]. Secondly, alterations of EG by factors other  
203 than the choice of the anesthetics cannot be ruled out - e.g. preoperative fluid status, the fluid amount  
204 given during surgery, types of fluids given, blood loss (in our study none of the patients required  
205 transfusion in the first two hours after the surgery), chronic diseases, usage of tourniquet and bone  
206 cement (in our study 9 patients out of 60) [30, 36, 37]. We presumed hip and knee surgery were  
207 comparable in the severity of surgical trauma. Thirdly, our study was not randomized, and patients  
208 entered the study in consecutive fashion at two different departments that may cause bias in patient  
209 selection and therefore affect study results.



210 Due to the key role of EG in humans in protecting the vascular endothelium against capillary  
211 leakage, inflammation, and coagulation, the injury of glycocalyx is expected to lead to an increased  
212 capillary permeability resulting in tissue edema, a proinflammatory environment, hypercoagulability  
213 and loss of vascular reactivity. Any of these mechanisms may contribute to the development of various  
214 postoperative non-surgical complications [3, 6, 38]. Limiting damage to the EG by using neuraxial  
215 blockade instead of general anesthesia may, therefore, serve as another piece of the puzzle in searching  
216 for an explanation why some authors report better outcome after the use of neuraxial anesthesia [26,  
217 39, 40]. In our opinion, our findings support the idea that EG and its damage/dysfunction may play a  
218 non negligible role in the clinical outcome of surgical patients. Searching for the “glycocalyx-friendly”  
219 anesthesia techniques in order to mitigate EG damage caused by surgery might, therefore, represent  
220 an important research area. Preconditioning the EG (e.g. pharmacologically) before major elective  
221 surgery may serve as a future target in perioperative medicine.

## 222 6. Conclusion

223 Elective joint replacement surgery led to significant changes of PBR dimensions indicating EG  
224 damage during the procedure. Undergoing the procedure under neuraxial blockade was associated  
225 with less EG damage compared to general anesthesia as measured by PBR. Preservation of the EG  
226 may be associated with a decreased rate of postoperative complications.

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229 Authors' contribution: David Astapenko acquired half of the data, collected all data, performed  
230 statistics, evaluated the results, was responsible for manuscript writing, editing and submission, images,  
231 graph, and tables preparation. Jiri Pouska acquired half of the data. Jan Benes was responsible for  
232 manuscript editing and evaluation of the results. Roman Skulec was responsible for manuscript editing.  
233 Christian Lehmann evaluated the results, was responsible for manuscript editing and language check.  
234 Hans Vink was responsible for manuscript editing. Vladimir Cerny set up the study protocol, calculated  
235 the size of the patients' cohorts, evaluated the results and was responsible for manuscript writing,  
236 editing, and submission.

237 All authors read and approved the final manuscript.

238 David Astapenko, MD, provided images of his sublingual microcirculation for illustration. Petra  
239 Hirsova, PhD, contributed to the final shape of the manuscript.

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