Effect of Intravenous Administration of Sodium-Lactate on Retinal Blood Flow in Healthy Subjects

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PURPOSE. The present study was designed to investigate the effect of intravenously administered sodium lactate on ocular blood flow.

METHODS. Twelve healthy male volunteers received either sodium lactate (0.6 mol/L) or physiologic saline solution in a randomized, double-masked, two-way crossover study. Sodium lactate or placebo were administered at an infusion speed of 500 and 1000 mL/h for 30 minutes each. Blood flow measurements were performed in the last 10 minutes of the infusion periods. Retinal blood flow was calculated based on the measurement of maximum erythrocyte velocity, assessed with bidirectional laser Doppler velocimetry, and retinal vessel diameter obtained with a retinal vessel analyzer. Choroidal blood flow was assessed with laser Doppler flowmetry and laser interferometric measurement of fundus pulsation amplitude.

RESULTS. Administration of lactate increased blood lactate concentration from 1.3 ± 0.4 to 3.9 ± 0.7 mmol/mL (P < 0.001) and to 7.1 ± 1.4 mmol/mL (P < 0.001) at infusion speeds of 500 and 1000 mL/h, respectively. At these blood lactate concentrations, retinal blood flow increased by 15% ± 20% and by 24% ± 37% (ANOVA, P = 0.01). Fundus pulsation amplitude increased by 3% ± 6% and 10% ± 5% (ANOVA, P = 0.04) at the two plasma lactate concentrations. Subfoveal choroidal blood flow measured with laser Doppler flowmetry tended to increase by 10% ± 15% and 13% ± 20% (ANOVA, P = 0.19), but this effect was not significant. Infusion of sodium lactate induced alkalosis in arterial blood taken from the earlobe (7.41 ± 0.03 at baseline; 7.50 ± 0.03 during lactate infusion; P = 0.001).

CONCLUSIONS. The data indicate that intravenously administered sodium lactate increases retinal blood flow. Whether this is related to a cytosolic redox impairment or to other hitherto unidentified mechanism remains to be clarified. Further studies are needed to determine whether lactate plays a role in regulation of choroidal blood flow. (Invest Ophthalmol Vis Sci. 2003;44:3972–3976) DOI:10.1167/iovs.02-1272

Based on observations in diabetic animals, it has been speculated that a cytosolic redox imbalance—namely, an increase of the intracellular ratio of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD) signals blood flow need in several tissues.1-5 According to this hypothesis, accumulation of electrons in cytosolic NADH, regardless of their origin, increases the cytosolic free NADH-to-NAD+ ratio and activates pathways to augment blood flow. It has been hypothesized that this redox imbalance develops during diabetic conditions,2-4 in hypoxic and ischemic as well as in working tissue,1 only differing in the source of electrons. Whether this hypothesis is correct is, especially in diabetic conditions, still a matter of controversy.5,6

To clarify whether a cytosolic redox impairment can alter ocular blood flow, the present study investigated the effect of increased free cytosolic NADH concentration on retinal and choroidal blood flow. Experimental modulation of the NADH-to-NAD+ ratio becomes possible, because a near equilibrium exists between cytosolic free NADH-to-NAD+ and intracellular lactate-to-pyruvate ratios, established by lactate-dehydrogenase.7 Furthermore, intracellular and extracellular lactate-to-pyruvate ratios are in near equilibrium, which is established by monocarboxylate transporters.8 Thus, intravenous administration of sodium lactate leads to an increase in the lactate-to-pyruvate ratio and drives reduction of the NADH-to-NAD+ ratio, which in turn increases intracellular NADH concentration.7 This method of evaluation and changing cytosolic NADH-NAD+ ratios has been used in several earlier animal experiments.1

Thus, the present study was performed to clarify whether an increase of cytosolic NADH evoked by intravenous administration of lactate may alter ocular blood flow. This could bring new insight into how blood flow is controlled in the human retina and choroid.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of Vienna University School of Medicine and followed the guidelines of Good Clinical Practice and the Declaration of Helsinki. Twelve healthy male nonsmoking subjects were included. Volunteers signed a written informed consent and passed a screening examination that included medical history and physical examination; 12-lead electrocardiogram; complete blood count; activated partial thromboplastin time; thrombin time; fibrinogen; clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and total protein); hepatitis-A, -B, -C and HIV-serology; and urinalysis. Subjects were excluded if any abnormality was found as part of the screening, unless the investigators considered an abnormality clinically irrelevant. An ophthalmic examination was performed in each subject before the study day. Inclusion criteria were normal ophthalmic findings, ametropia of less than 3 D, and anisometropia of less than 1 D. In all subjects, the right eye was studied.

Experimental Paradigm

Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial days and were studied after a light breakfast. The study was performed in a double-masked, placebo-controlled, randomized, two-way crossover design on two study days.
A resting period of at least 20 minutes was scheduled for all subjects. After stable hemodynamic conditions had been achieved and ensured by repeated blood pressure measurements, baseline readings of all outcome parameters were performed. Thereafter, subjects received either sodium lactate (0.6 mol/L; Mayrhofer Pharmazeutika, Linz, Austria) in two stepwise increasing infusion speeds (500 and 1000 mL/h) or placebo (physiological saline solution), for 50 minutes each. During the last 10 minutes of the infusion periods, blood flow measurements were performed, and blood samples were obtained. On the second study day, the same time schedule was followed, and subjects crossed over to the alternative treatment. A washout period of at least 3 days was allowed between the two study days.

Retinal Vessel Analyzer

The retinal vessel analyzer (RVA; Zeiss, Jena, Germany) is a commercially available system that comprises a fundus camera (FF 450, Zeiss), a video camera, a high-resolution video recorder, a real-time monitor, and a personal computer with vessel diameter-analyzing software. The RVA allows the precise determination of retinal vessel diameter with a time resolution of 25 readings per second. The fundus was illuminated with light in the range of wavelengths between 567 and 587 nm. In this spectral range, the contrast between retinal vessels and the surrounding tissue is optimal. Retinal irradiance was approximately 220 μW · cm⁻², which is approximately 50 times lower than the maximum level allowed for constant illumination of the retina at the wavelengths mentioned earlier. The system provides excellent reproducibility and sensitivity.9 In the present study, major temporal arteries and veins were studied. Measurements of retinal vessel diameters were taken between 1 and 2 disc diameters from the margin of the optic disc.

Laser Doppler Velocimetry

The principle of red blood cell velocity measurement by laser Doppler velocimetry (LDV) is based on the optical Doppler effect. Laser light, scattered by moving erythrocytes, is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte velocity (V_max).10 With bidirectional LDV, the absolute velocity in the retinal vessels can be obtained.10,11 In the present study, we used a fundus camera-based system with a single-mode laser diode at a center wavelength of 670 nm (model 4000; Oculix Sarl; Arbaz, Switzerland). The Doppler shift power spectra were recorded simultaneously for two directions of the scattered light. The scattered light was detected in the image plane of the fundus camera. This scattering plane can be rotated and adjusted in alignment with the direction of V_max, which enables absolute velocity measurements. Red blood cell velocity was measured at the same locations as retinal vessel diameters by using bidirectional LDV.

Calculation of Retinal Blood Flow

Retinal blood flow in single retinal veins was calculated based on the measurements of maximum erythrocyte velocity (V_max) assessed with LDV and retinal vessel diameter (D) obtained with the RVA. From V_max, mean blood velocity in retinal veins (V_mean) can be calculated as V_mean = V_max/1.6. This relation has been found by experiments involving blood flow in glass tubes with a diameter in the range of those measured in our study.12,13 With the use of this relation, mean velocities in retinal veins can be obtained. Blood flow (Q) through a specific retinal vessel was then calculated as

\[ Q = (V_{\text{max}}/1.6) \cdot (\pi \cdot D^2/4) \]

Laser Doppler Flowmetry

Choroidal blood flow was assessed with a fundus-camera–based laser Doppler flowmeter (model 4000; Oculix Sarl) introduced by Riva et al.13,14 The principles of LDF have been described in detail elsewhere.15 Briefly, the vascularized tissue is illuminated by coherent laser light. Scattering on moving blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast, static scattering in tissue does not change light frequency but leads to a randomization of light directions impinging on the RBCs and consequently a broadening of the spectrum of scattered light (Doppler shift power spectrum, DSPS). From the DSPS the mean RBC velocity, the blood volume, and the blood flow can be calculated in relative units. The laser beam was directed to the fovea to assess blood flow in the submacular fovea.

Fundus Pulsation

Pulse synchronous pulsations of the ocular fundus were assessed by a laser interferometric method, described in detail by Schmetterer et al.16 The fundus pulsation amplitude (FPA), representing the maximum distance change of distance between cornea and retina during the cardiac cycle, gives an estimate of pulsatile blood flow on the selected fundus location.17,18 For this purpose, the ocular fundus is illuminated by a high-coherence laser beam (λ = 785 nm) along the optical axis with a laser power of approximately 80 μW. The laser light is reflected at both the retina and the outer surface of the cornea, the latter serving as a reference wave. The relative change in distance between cornea and retina during a cardiac cycle may be evaluated by analyzing the interference fringes that are produced by the two reemitted waves. To assess pulsatile choroidal blood flow, measurements in the present study were performed in the macula.

Measurement of Blood Lactate Levels

Blood lactate values were measured with a reflectance photometer (Accusport; Roche Diagnostics, Inc., Mannheim, Germany) from blood samples drawn from the carotid. The values obtained by the Accusport have been shown to be comparable in accuracy, linearity, and reliability with those obtained by a laboratory reference method.19,20

Blood Pressure Measurement

Systolic, diastolic, and mean blood pressures were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded from a finger pulse oximetric device. Measurements were performed before, during, and after flicker stimulation.

Blood Gas Analysis

Blood gas levels were determined from capillary blood samples obtained from the carotid. After a paste was applied to the carotid to induce capillary vasodilatation (Finalgon; Thomae, Biberach, Germany), a lancet incision was made. The arterial blood was taken into a thin glass capillary tube. Arterial pH, pCO2 and pO2 were determined with an automatic blood gas analysis system (995-Hb; AVL, Graz, Austria). This technique accurately measures arterial blood gas tensions.21

Statistics

Data are presented as means ± SD. Repeated-measures ANOVA was used to assess blood flow effects of lactate versus placebo. Student's paired t tests were used for post hoc analysis. P < 0.05 was considered as the level of significance. Calculations were performed on computer (Statistica software; Statsoft, Tulsa, OK).

RESULTS

Infusion of sodium lactate was well tolerated by all subjects. Baseline levels of hemodynamic and ocular blood flow parameters are summarized in Table 1. Mean arterial pressure (MAP) and intraocular pressure (IOP) were not affected by either lactate or placebo infusion (Table 2) and were comparable on both study days.

Plasma Lactate and Blood pH Values

As shown in Figure 1, administration of sodium lactate increased plasma lactate concentrations from 1.3 ± 0.4 mmol/L

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Arterial vessel diameter (μm) 122 ± 14, 123 ± 14
Venous vessel diameter (μm) 152 ± 15, 157 ± 14
FPA (μm) 4.8 ± 1.6, 4.7 ± 1.6
Blood pH 7.39 ± 0.04, 7.41 ± 0.03
Blood lactate concentration (mmol/L) 1.6 ± 0.5, 1.4 ± 0.4

Baseline parameters were obtained during the 10 minutes before infusion. The averaging period for hemodynamic measurements with LDF and LDV was 120 seconds. Results are presented as the mean ± SD (n = 12). Choroidal blood flow (ChBF) was assessed with LDF, red blood cell velocity was assessed with bidirectional LDV.

Retinal Blood Flow
Placebo had no effect on red blood cell velocity, retinal vessel diameter, or retinal blood flow. Infusion of lactate increased red blood cell velocity by 16.3% (P = 0.029, ANOVA) at the two doses administered. Arterial vessel diameter tended to increase from 123 ± 14 to 125 ± 16 and 127 ± 16 μm (P = 0.1, ANOVA) and venous vessel diameter from 152 ± 15 to 152 ± 16 and 154 ± 16 μm (P = 0.2, ANOVA) at the two infusion speeds, respectively. Thus, calculated retinal blood flow increased after administration of lactate by 15% ± 20% and 24% ± 37%, respectively (P = 0.01, ANOVA).

Choroidal Blood Flow
FPA measured with laser interferometry increased after administration of sodium lactate by 3% ± 6% and 10% ± 5% (P = 0.04, ANOVA) at the two administered doses. Subfoveal choroidal blood flow assessed with LDF tended to increase by 10% ± 15% and 13% ± 20%, but this effect failed to reach level of significance (P = 0.19, ANOVA). Placebo did not affect FPA or choroidal blood flow.

Discussion
In this study, we present evidence that intravenous administration of sodium lactate can alter retinal blood flow in healthy volunteers. This is in keeping with recent animal experiments revealing that intravenous administration of lactate leads to increased blood flow of 50% in the rat retina. It has been shown that this blood flow increase evoked by administration of lactate is reversible by the administration of pyruvate, which can shift the NADH-to-NAD⁺ ratio toward NAD⁺. It has recently been hypothesized that increases in blood flow during hyperglycemia, muscle work, and other conditions are all mediated through essentially the same signaling cascade. According to this hypothesis, the latter physiologic and pathologic conditions should all end up in increased cytosolic NADH concentration, differing only in the source of electrons that lead to this redox impairment. Increased glycolysis during work, for example, can alter the NADH-to-NAD⁺ ratio, because the transfer of electrons exceed their use for mitochondrial ATP synthesis, which in turn leads to an increase of NADH. The same mechanism holds true for hypoxia, in which insufficient reoxidation augments NADH concentrations during tissue hypoxia.

Further evidence from pathologic conditions, namely hyperglycemia, also seems to support this hypothesis: Increased flux through the sorbitol pathway could account for cytosolic redox impairment and has been blamed to induce increased blood flow during hyperglycemia as well as diabetic long-term complications including neuropathy, retinopathy, and nephropathy. Both, the increase in NADH and augmented blood flow have been shown to be reversible by administration of inhibitors of the sorbitol pathways in animal experiments. In contrast, inhibitors of sorbitol pathway failed to show effects in clinical trials in humans. The reason for this failure is currently unclear, but may be related to the low dose of sorbinil used in these clinical trials.

Alternatively, a change in tissue pH could be responsible for the increase in retinal blood flow in the present study. It has been shown in a variety of studies that pCO₂, an important determinant of pH is a regulator of retinal and choroidal blood flow. However, after administration of lactate an increase in blood pH has been observed. Whether this increase in blood pH also reflects tissue pH is unclear, because specific lactate transporters exist in the human retina. Previous studies of the effect of lactate on blood pH are inconsistent. Ido et al. reported an increase in blood pH after lactate infusion, which is in keeping with the results of the present study. By contrast, in another study blood and tissue acidosis were observed in the retina after lactate infusion in the miniature pig. In the present study we did not measure tissue pH, because there is a lack of adequate techniques currently available for measuring retinal pH in vivo. Thus, we cannot prove whether intravenous administration of sodium lactate leads to local tissue acidification or tissue alkalosis as a result of generalized alkalosis.

Furthermore, it has been shown in animal experiments that blood flow increases during changes in plasma lactate concentrations are pH independent. In fact, two lines of experimental evidence strongly suggest that the blood flow increase during lactate infusion is not mediated by changes in tissue pH.

Table 2. Hemodynamic Parameters, IOP, and Blood pH at Baseline and after Administration of placebo or Lactate

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Lactate (0.6 mol/L)</th>
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<tbody>
<tr>
<td></td>
<td>500 mL/h</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>80 ± 9</td>
</tr>
<tr>
<td>Pulse rate (bpm)</td>
<td>74 ± 12</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.59 ± 0.04</td>
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</table>

Results are presented as the mean ± SD (n = 12).
* Denotes values significantly different from the baseline values (P < 0.05, t-test).
First, it has been shown that injection of both acid and neutral solution of L-lactate, causes similar vasodilatating effects in the retina. Second, investigators in two studies report that only L-lactate, not D-lactate, leads to blood flow increases in animal experiments. The reason for the latter effect has yet to be clarified. One could hypothesize that the absence of an effect of D-lactate is based on a strict stereospecificity of lactate transporter mechanisms in the cell membrane.

According to our data, changes in retinal blood flow evoked by infusion of lactate is attributable mainly to increased blood velocity and not to changes in retinal vessel diameters. Although we observed a tendency of retinal vessel diameters to increase, this effect did not reach a level of significance, indicating only small changes below our limit of detection (3% for retinal veins and 4% for retinal arteries). This is compatible with the results of Brazitikos et al. showing that systemic administration of lactate had no influence on retinal arterial diameters in an animal study. Thus, changes in retinal blood flow evoked by administration of lactate can be attributed mainly to increased blood velocity. This indicates that much of the blood flow response occurs within the small arterioles, which are the major sites of resistance to flow. A systemic contribution to the increase in retinal blood flow during lactate infusion is unlikely, because neither blood pressure nor IOP were changed. Moreover, the effect of lactate in the choroid was smaller than that in the retina.

Pulsatile choroidal blood flow assessed with laser interferometry significantly increased after administration of lactate. Subfoveal choroidal blood flow measured with LDF also tended to increase, but this effect failed to reach the level of significance. This difference in statistical significance may be related to the better reproducibility of the laser interferometric method. Alternatively, the results of the present study may indicate a change in choroidal blood flow pulsatility, because FPA is only a measure of the pulsatile component of blood flow in the choroid. Further studies are required to clarify whether lactate may play a role in choroidal blood flow regulation in humans.

In conclusion, we present evidence that retinal blood flow is sensitive to changes in blood plasma lactate concentrations after administration of sodium lactate. Whether this increase is related to a change in the cytosolic NADH-to-NAD⁺ ratio or to other mechanisms induced by administration of lactate has yet to be clarified.

References
4. Van den Enden M, Nyengaard J, Ostrow E, Burgan J, Williamson J. Elevated glucose levels increase retinal glycolysis and sorbitol