**Blood–Retinal Barrier Glycerol Permeability in Diabetic Macular Edema and Healthy Eyes: Estimations from Macular Volume Changes after Peroral Glycerol**

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**PURPOSE.** To compare the changes in macular volume (MV) between healthy subjects and patients with diabetic macular edema (DME) after an osmotic load and to determine the glycerol permeability ($P_{\text{gly}}$) of the blood–retinal barrier (BRB).

**METHODS.** In this unmasked study, 13 patients with DME and 5 healthy control subjects ingested a glycerol solution (0.57 g/mL) of 3 mL/kg body weight (maximum, 250 mL). Subsequently, the MV determined by the retinal maps provided by the optical coherence tomography (OCT) fast macular thickness protocol was monitored at 12 time points for 180 minutes. A mathematical model of glycerol and osmotic water movement across the BRB was constructed to estimate $P_{\text{gly}}$.

**RESULTS.** Median MV decreased from 7.30 mm$^3$ (range, 6.68–7.55) to the maximum median ΔMV of $-0.30$ mm$^3$ (25%–75% quartile: $-0.34$ to $-0.25$) in the healthy volunteers and from 7.97 mm$^3$ (range, 6.85–9.89) to ΔMV of $-0.14$ mm$^3$ (25%–75% quartile: $-0.19$ to $-0.08$) in the diabetic group (intergroup difference: $P < 0.05$). $P_{\text{gly}}$ was $6.1 \times 10^{-6}$ (SE $1.8 \times 10^{-6}$) and $74 \times 10^{-6}$ (SE $42 \times 10^{-6}$) cm/s in the healthy and diabetic participants, respectively ($P < 0.0001$). No rebound phenomenon was observed in either group.

**CONCLUSIONS.** The maximum reduction in MV was doubled in the healthy group compared with the diabetic group, whereas the glycerol permeability was 12 times higher in the diabetic participants. These findings confirm the paradigm of BRB breakdown in DME, but also suggest a novel procedure for the determination of retinal permeability to various agents, which is independent of the vitreous condition (ClinicalTrials.gov number, NCT00353671). 

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The normal blood–retinal barrier (BRB) restricts the free movement of hydrophilic molecules such as glucose and amino acids, as well as ions as small as sodium$^{1,2}$. In diabetic retinopathy, it is well accepted that the BRB leaks protein and fluid due to a breakdown of the barrier integrity.$^{3–8}$ Quantification of the barrier’s permeability to substances of different molecular structure and weight, and lipid solubility assists in understanding the mechanisms behind the deterioration of the barrier. Although the water permeability of the BRB has not been measured, it is thought to be substantial. An osmotic water permeability ($L_w$) of approximately 0.0004 cm/s/Osm has been demonstrated in a part of the outer BRB in frogs.$^9$ Hence, the water permeability of the BRB is thought to be at least 0.0001 cm/s/Osm.

By vitreous fluorometry, BRB permeability to fluorescein, a small molecule with a molecular weight of 376, has been found to be increased by a factor of 12 in patients with diabetic macular edema (DME) when compared with healthy control subjects.$^7$ The most commonly used osmotic agents for therapeutic purposes, glycerol and mannitol, are smaller than fluorescein, but data on the permeability of the BRB to these substances are lacking in both healthy and diabetic persons.

The osmotic property of these molecules relies on the presence of a barrier between the vascular system and the tissue to be dehydrated. For optimal effect, the barrier has to be selectively permeable to water, to drive the osmotic water movement across the barrier, whereas it should be, at least partly, impermeable to the osmotic drug. Therefore, the permeability of the BRB to glycerol is predictably much less than it is to water.

The administration of glycerol is assumed to initiate an osmotic water movement from the retina to the blood. The osmotic effect diminishes accordingly, which is reinforced by the accumulation of glycerol in the retina due to a relatively slower diffusion of glycerol into the retinal tissue. During the systemic clearance of glycerol, a point is reached at which the retinal concentration of the osmotic agent exceeds the level in the blood, resulting in an undesired rebound phenomenon characterized by a reverse osmotic water transport from the blood to the retina. As a consequence of the generally increased permeability, the rebound effect is assumed to be more pronounced in DME than in the normal retina.

The water movements result in changes in retinal volume that can be measured by optical coherence tomography (OCT). We therefore conducted a prospective, comparative study to monitor the effects of peroral glycerol on the OCT-based macular volume in normal subjects and in patients with DME. In particular, a mathematical model based on a simple two-compartment assumption of the transport of glycerol and water across the BRB was produced, incorporating the volume data and the changes in plasma osmolarity to estimate the BRB’s permeability to glycerol in diabetic persons relative to that in healthy control subjects.

**METHODS**

Fifteen diabetic patients with DME were recruited from our outpatient department, in addition to five healthy volunteers. All participants...
were ≥18 years of age and had a systemic blood pressure (BP) of ≤160/90 mm Hg (mean of three measurements with a few minutes of rest between). Patients with type 1 or 2 diabetes were eligible, whereas the control subjects, to their own knowledge, were healthy. Nonocular exclusion criteria in both groups were pregnancy and moderate to severe cardiac and/or pulmonary insufficiency defined as a minimum of two of the following symptoms: crural edemas, bilateral basal lung crackles by auscultation, and low-activity-induced dyspnea judged by the investigator. Participants with plasma creatinine >300 μM were also excluded.

Ocular inclusion criteria were best corrected visual acuity of at least 70 letters on the ETDRS chart at a 4-m distance in the healthy group and ≥60 letters on the ETDRS chart at a 4-m distance in the diabetic group, to obtain satisfactory fixation during the OCT scanning process. Additional inclusion criteria for the diabetic group were nonproliferative diabetic retinopathy (ETDRS diabetic retinopathy level, ≥32 and ≤53) and macular edema defined as retinal thickness of ≥250 μm in the foveal region or a minimum area of edema of one disc area within 3000 μm² from the fovea based on slit lamp biomicroscopy and subsequent OCT analysis. Eyes with other macular diseases, glaucoma, or prior laser photoagulation treatment of any kind were excluded.

In case of the eligibility of both eyes, the eye with the most central involvement was defined as the primary eye in the diabetic group, and in the healthy volunteers, the primary eye was chosen randomly. The project was approved by the Scientific-Ethics Committee of the County of Copenhagen and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained after explanation of the nature and possible consequences of the study and before the first study-related examination for each participant.

**Examination Procedures**

Baseline examinations in both groups comprised visual acuity testing by the ETDRS guidelines and slit lamp biomicroscopy, including measurement of intraocular pressure withplanation tonometry. Mydriasis was induced with phenylephrine hydrochloride (10%) and tropicamide (1%).

The size and location of the edema in the diabetic group were confirmed by the OCT fast macular thickness protocol. As indirect indicators of renal function, plasma sodium, potassium, and creatinine were determined. HbA1c confirmed the metabolic state.

After visual acuity testing and the induction of mydriasis, the subjects received a glycerol solution (0.57 g glycerol/mL) administered personally as a dose of 3 mL solution/kg patient body weight. The maximum dose was set at 250 mL except for the 267 mL given to one diabetic patient who weighed 89 kg. To facilitate swallowing, the participants were allowed to drink up to 300 mL tap water.

After the initial 20 minutes and during the succeeding intervals between the scanning sessions, the patients were permitted to walk around in the department. Diabetic subjects were also allowed to eat and drink small meals if needed, but insulin injections were kept to a minimum. The capillary glucose (CG) was monitored at baseline, 90, and 120 minutes (Precision Xceed; Abbott Laboratories, Alameda, CA), and the systemic blood pressure at 0, 5, 10, 20, 30, 60, 120, and 180 minutes. The mean arterial blood pressure was calculated as diastolic pressure + 1⁄3(systolic – diastolic blood pressure).

**OCT Procedures**

Macular volume (MV) was assessed by optical coherence tomography (Fast Macular Thickness Map protocol, Stratus OCT, ver. 4.01; Carl Zeiss Meditec, Inc., Dublin, CA) before and 2, 4, 8, 10, 15, 20, 30, 60, 90, 120, and 180 minutes after the last swallow of glycerol. The protocol constitutes six 6-mm radial lines arranged in a spoke pattern centered at the fovea. Each scan session began between 9 AM and 12 PM, at least 2 hours after the person had arisen from bed. All scans were performed by an experienced investigator (DNT).

A minimum of three fast macular thickness scans were obtained at each time point. Only those scans with a standard deviation (SD) of <15% of the center point thickness were accepted. Afterward, those scans per time point with the lowest SD of <15%, with the best fixation and without visible artifacts on the retinal maps were identified, to form a set. The remaining scans were excluded. Finally, for each OCT set, the mean MV was calculated from the three included scans by using the total MV values defined by the automatic algorithm (Stratus; Carl Zeiss Meditec, Inc.) and presented on the scan summary chart. These MV estimates result from multiplication of the circular scan area covering 0.283 cm² [π × (0.3 cm)²] and the average retinal height of this area. No masking was used for any of the study procedures.

The fast protocol was preferred to the macular thickness protocol, because it is far less time consuming, which was a crucial factor in the first 20 minutes of the examination period due to the repetitive scan pattern. The two protocols were quantitatively comparable in all nine OCT regions.

**Blood Samples**

A detailed description of all the blood sample analyses is presented in our previous paper.11 Plasma osmolarity (pOsm) was monitored before and at 5, 15, 30, 60, 120, and 180 minutes after glycerol intake. During the 3-hour examination period, these samples were stored at 4°C followed by immediate centrifugation, and pOsm was determined by freezing-point depression of the plasma (Osmomat 030; Gonotec GmbH, Berlin, Germany). If not analyzed right away, the plasma was stored at 4°C for a maximum of 10 days before analysis.

**Model**

The interpretation of the clinical data was based on a simplified two-compartment model describing the movements of water and glycerol between the blood vessels and the retinal space across the retinal endothelium (Fig. 1).

The osmotic activity of glycerol in the blood at time (t) after glycerol ingestion, Cb, gly(t), has been described elsewhere.11 Within the first 180 minutes after ingestion of an oral dose of glycerol (3 mL/kg), Cb, gly(t) could be described by a simple two-compartment model:

\[ C_{b,\text{gly}}(t) = C_{b,\text{gly}}(0) + B \cdot \left[ \exp(-k_1 \cdot t) - \exp(-k_2 \cdot t) \right] \]  \hspace{1cm} (1)

where \( C_{b,\text{gly}}(0) \) is the baseline plasma osmolarity, \( B \) is directly related to the maximum \( C_{b,\text{gly}} \), and \( k_1 \) and \( k_2 \) are the absorption and elimination constants for the osmolarity curve. Apart from a small difference in baseline osmotic activity in the blood, we found no difference in the kinetic parameters (\( B, k_1, \) and \( k_2 \)) between the diabetic and the healthy.
groups. $B$ was estimated to be 35 mOsml/L (SEM 66); $k_h$, 0.015 minute$^{-1}$ (SEM 0.013); and $k_w$, 0.006 minute$^{-1}$ (SEM 0.007), and the estimations were used in the following calculations.

The flux of glycerol from the blood across the BRB and into the retina at time $t$, $J_{brb}(t)$, is described by Fick’s law:

$$J_{brb}(t) = P_{brb} \cdot \left[ C_{brb}(t) - C_{brb}(0) \right]$$

where $C_{brb}(t)$ is the retinal osmotic activity of glycerol at time $t$, and $P_{brb}$ is the permeability of the BRB to glycerol. Furthermore:

$$C_{brb}(t) = \int J_{brb}(t) \cdot b^{-1} \cdot dt$$

where $b$ is the surface-to-volume ratio (i.e., the height or thickness) of the macula. By inserting equation 1, we obtain the differential equation for $C_{brb}(t)$:

$$\frac{d C_{brb}}{dt} = P' \cdot \left[ C_{brb}(0) + B \cdot \exp(-k_1 \cdot t) - \exp(-k_1 \cdot t) \right] - C_{brb}(t)$$

Equation 4 has the following analytical solution:

$$C_{brb}(t) = P' \cdot B \cdot \left( \frac{\exp(-k_2 \cdot t)}{P' - k_2} - \frac{\exp(-k_1 \cdot t)}{P' - k_1} \right) + \frac{(k_1 - k_2) \cdot \exp(-P' \cdot t)}{(P' - k_1) \cdot (P' - k_2)}$$

The volume flux across the BRB, $J_{vbl}$, is described by simple osmosis:

$$J_{vbl}(t) = L_p \cdot \left[ C_{vbl}(0) - C_{vbl}(t) \right] + E \cdot \Delta b(t)$$

where $L_p$ represents the osmotic water permeability of the BRB, and $E$ is the pseudoelasticity of the retinal tissue that is assumed to counteract the volume changes in a linear fashion. $\Delta b$ is the change in retinal volume per unit retinal surface area and can be interpreted as the average change in retinal thickness. Again, in equation 6, minute changes in $C_{vbl}$ due to volume changes are ignored. Because the integrated flux equals the volume change, the integration of equation 6 leads to the following differential equation with regards to $\Delta b$:

$$\frac{d \Delta b}{dt} = -L_p \cdot \left[ C_{vbl}(0) - C_{vbl}(t) \right] + E \cdot \Delta b(t)$$

When equation 5 is inserted into equation 7, a differential equation for $\Delta b(t)$ results, which has the analytical solution:

$$\Delta b(t) = C_1 \cdot \exp(-k_2 \cdot t) + C_2 \cdot \exp(-k_1 \cdot t) + C_3 \cdot \exp(-P' \cdot t) + C_4 \cdot \exp(-E \cdot L_p \cdot t)$$

where

$$C_1 = \frac{L_p \cdot B \cdot k_2}{(E \cdot L_p - k_2) \cdot (P' - k_2)}$$

$$C_2 = -\frac{L_p \cdot B \cdot k_1}{(E \cdot L_p - k_1) \cdot (P' - k_1)}$$

Equation 8 yields the functional relationship between the MV changes $\Delta b$, $L_p$, $P'$, and $E$ and the pharmacokinetic parameters $B$, $k_1$, and $k_2$ and time. When $\Delta MV$ is the change in MV in cubic millimeters, $\Delta b$ is the change in the surface to volume in centimeters, and the scanned area covers 0.283 cm$^2$, as outlined earlier in the OCT section, then $\Delta b$ is related to the measured changes in MV by $\Delta MV = 283 \times \Delta b$.

**Data Analysis**

Two diabetic participants were excluded post hoc from statistical analysis, because the most of their scans contained significant artifacts secondary to unexpected low signal strength in one patient and a steep, dome-shaped central edema in the other. Comparisons of the baseline characteristics between the 5 healthy and 13 diabetic participants were performed with Student’s $t$-tests except for the ratios of the sexes, which were analyzed manually with a $z$-test. (All differences were normally distributed, but for capillary glucose, HbA1c, and baseline MV, logarithmic transformation of the raw data was performed to correct for the difference in variance between the two groups.)

When the effect of glycerol on the retina was calculated, MV (i.e., the averaged total MV of three scans per time point) was used as the primary parameter. Overall group comparisons of MV between the two study groups were analyzed by the Mann-Whitney rank sum test, as the MV was only borderline normally distributed, with some variance inhomogeneity.

We were unable to identify a transformation, which resulted in fully normally distributed residuals for ANOVA of the entire data set from 0- to the 180-minute time point. Nevertheless, as the residuals were borderline normally distributed, we performed a two-way, repeated-measures ANOVA on $\Delta MV$ to examine any interaction between time and the presence of diabetes. Subsequently, the complete dataset was divided into an early and a late phase (0-10 minutes and 15-180 minutes, respectively) with separate analysis of the datasets by linear regression model, where the parameter $\Delta MV$ depended on the presence of diabetes. The practical “time resolution” of 2 minutes for the scan sequences within the first 10 minutes was the shortest feasible with the present study design. Hence, the cutoff point of the initial phase at 10 minutes was a compromise between the need for at least four time points after baseline to produce reliable time curves and to distinguish the two study groups’ different rates of extravasation of glycerol setting in shortly after the ingestion. For both the early and the late phases, the residuals of the changes in MV were normally distributed (Kolmogorov-Smirnov test; $P > 0.2$).

Within-group differences of the MV changes from baseline were analyzed with Wilcoxon signed rank test.

For each time point and each group (diabetic or healthy controls), the curve-fitter function of a commercial software program (SigmaPlot, ver. 10.0; Systat Software, Chicago, IL) was used to fit equation 8 to the $\Delta MV$ values. By the sum of square derivations (SSDs) in the computer analysis output, $F$-tests were performed for reductions in the number of parameters in the model. Analysis of the residuals from the curve-fitting procedure confirmed that the averages of $\Delta MV$ were normally distributed. A significance level of 0.05 was used. All results are presented without correction for mass significance.

**Results**

Baseline clinical characteristics of both the diabetic and the healthy participants are listed in Table 1. As expected, significant differences were found in visual acuity ($P = 0.013$; $t$-test), HbA1c ($P = 0.017$), and capillary glucose ($P = 0.035$). Otherwise, the healthy and the diabetic subjects were generally...
comparable, except that the healthy group was younger than the diabetic group ($P = 0.032$).

### Macular Volume

Table 2 summarizes the changes in MV after glycerol administration. About half the macular edemas in the diabetic group were predominantly of focal character, whereas the rest were more diffusely distributed.

Throughout the examination period, the MV in the diabetic patients was higher than that in the healthy subjects ($P < 0.001$, Mann-Whitney test).

The time course of the changes in MV from baseline ($\Delta$MV) differed between the diabetic and the healthy groups. Figure 2 is a presentation of illustrative OCT scans from a healthy and a diabetic participant.

Two-way, repeated-measures ANOVA on MV confirmed a significant interaction between time and the presence of diabetes ($P < 0.01$). The results of the subsequent linear regression analyses of early- and late-phase changes in MV from baseline (0–10 minutes and 15–180 minutes, respectively) are shown in Figure 3 for each study group. For the early measurements, no statistically significant effect of diabetic status existed ($P > 0.3$), whereas time was a significant factor ($P = 0.02$). The opposite results were found for the late phase: a statistically significant effect of diabetes ($P < 0.001$), but not of time ($P > 0.1$), on $\Delta$MV.

Overall, a significant difference was found in the median maximum effect: $-0.14 \text{ mm}^3$ (25%–75% quartile: $-0.19$ to $-0.08$) and $-0.30 \text{ mm}^3$ (25%–75% quartile: $-0.34$ to $-0.25$) in the diabetic and healthy groups, respectively ($P = 0.038$ for the difference, Mann-Whitney rank sum test).

Four diabetic subjects and one healthy one showed a clear rebound phenomenon over time, and four persons in the diabetic group had a slight rebound effect. As the ranges in MV in Table 2 indicate, it had a tendency to occur earlier in the monitoring period in the diabetic subjects compared with the healthy ones. Nevertheless, in neither group was significant rebound documented for the whole group ($P > 0.13$, Wilcoxon signed rank test).

### Estimation of the BRB Permeability to Glycerol

A mathematical model of the fluid and glycerol movements was deduced as described in the Methods section. We used the pharmacokinetic data for the osmotic activity representing the glycerol concentration in blood obtained from the same group of patients as presented previously.

Figure 4 shows the fitting of the function defined by equation 8 to the changes in MV for each time point and for each group. The parameter $L_p$ (i.e., the osmotic water permeability) could not be fitted because the SSDs did not have a minimum when the parameter was varied.

Since the function defined by equation 8 was only minimally affected by changes in $L_p$ above 0.001 cm/s/Osm, this observation was expected. Figure 5 shows the respective SSDs when the model was fit with different fixed values of $L_p$. The SSD rapidly increased once $L_p$ obtained a value below 0.001 cm/s/Osm. A fixed value of $L_p$ of 0.001 cm/s/Osm was therefore used to estimate the two other parameters $P'$ and $E$.

The estimate of $P'$ was $2.4 \times 10^{-4}$ seconds$^{-1}$ (SE 0.7 $\times 10^{-4}$) and $26 \times 10^{-4}$ seconds$^{-1}$ (SE 15 $\times 10^{-4}$) for the healthy and diabetic groups, respectively, corresponding to a significant difference between groups ($P < 0.0001$, $F$-test). The common elasticity parameter $E$ was estimated to be 18 seconds$^{-1}$ (SE 3). On the basis of the baseline MV measurements listed in Table 2 and the relation $P_{gly} = P' - b$ (see the Methods section), the BRB permeability to glycerol ($P_{gly}$) was found to be $6.1 \times 10^{-6}$ cm/s (SE 1.8 $\times 10^{-6}$) in the healthy control group and $7.4 \times 10^{-6}$ cm/s (SE 42 $\times 10^{-6}$) in the diabetic group.

### Discussion

In the present study, we demonstrated that the MV decreased after induction of plasma hyperosmolarity in participants with DME and in healthy subjects. Thus, the retina behaves as an osmometer, and the initial response was similar in the diabetic and healthy subjects. However, the retinal volume returned to...

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**Table 1.** Baseline Characteristics Presented as the Mean (SD) and for Log-Transformed Parameters as the Mean (95% CI)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy ($n = 5$)</th>
<th>Diabetes ($n = 13$)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female ratio</td>
<td>2/3</td>
<td>5/8</td>
<td>0.95</td>
</tr>
<tr>
<td>Age, y</td>
<td>55 (10)</td>
<td>65 (7)</td>
<td>0.032</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69 (14)</td>
<td>82 (16)</td>
<td>0.15</td>
</tr>
<tr>
<td>Diabetes type 1/type 2</td>
<td>0/0</td>
<td>0/13</td>
<td></td>
</tr>
<tr>
<td>Time since diagnosis, y</td>
<td>—</td>
<td>16 (8)</td>
<td></td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.6 (4.5–7.0)</td>
<td>7.1 (5.0–10.0)</td>
<td>0.017</td>
</tr>
<tr>
<td>Capillary glucose, mM</td>
<td>5.5 (4.1–7.2)†</td>
<td>10.0 (4.4–20.2)</td>
<td>0.055</td>
</tr>
<tr>
<td>P-creatinine, µM</td>
<td>82 (24)</td>
<td>89 (27)</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mm Hg</td>
<td>99 (8)</td>
<td>89 (14)</td>
<td>0.56</td>
</tr>
<tr>
<td>Visual acuity, primary eye, ETDRS letters</td>
<td>89 (11)</td>
<td>75 (9)‡</td>
<td>0.013</td>
</tr>
</tbody>
</table>

* Based on $t$-tests for HbA1c, capillary glucose, and MV after log transformation.
† $n = 3$.
‡ The Snellen equivalent of 75 letters is 20/32.

**Table 2.** Changes in MV after Ingestion of Glycerol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Healthy ($n = 5$)</th>
<th>Diabetes ($n = 13$)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.30 (6.68–7.35)</td>
<td>7.97 (6.85–9.89)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.30 (6.75–7.41)</td>
<td>7.88 (6.83–9.89)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.21 (6.73–7.35)</td>
<td>7.88 (6.88–9.89)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.21 (6.62–7.37)</td>
<td>7.89 (6.88–9.91)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.12 (6.65–7.23)</td>
<td>7.85 (6.82–9.88)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>7.10 (6.64–7.22)</td>
<td>7.83 (6.96–9.84)*</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>7.08 (6.58–7.17)</td>
<td>7.78 (6.88–9.86)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.25 (6.57–7.29)</td>
<td>7.78 (6.77–9.91)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>7.00 (6.57–7.02)</td>
<td>7.85 (6.82–10.04)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>7.02 (6.79–7.43)</td>
<td>7.89 (6.80–10.21)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>7.13 (6.59–7.52)</td>
<td>7.90 (6.73–10.26)</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>7.16 (6.55–7.39)</td>
<td>7.98 (6.85–10.19)</td>
<td></td>
</tr>
</tbody>
</table>

Glycerol (0.57 g/mL) was administered at 3 mL/kg body weight: median (range).

* $n = 12$. 

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baseline within 1 hour in the diabetic group, whereas it remained decreased in the healthy group throughout the 3-hour observation period.

Animal studies have shown a similar osmometer-like effect of the outer BRB (i.e., the retinal pigment epithelium; RPE). Negi and Marmor in 1984 found that the osmotic concentration of iatrogenous subretinal fluid was less important for the resorption rate than was the permeability of the “membrane” (RPE) to the osmotic substances in rabbits. This observation was explained from the momentary equilibrium between the

**FIGURE 2.** Representative OCT B-scans and respective thickness maps from a healthy (left) and a diabetic (right) participant at 0 (top), 30, 90, and 180 (bottom) minutes after ingestion of glycerol. For the healthy person, the corresponding MV at baseline was 7.22 mm³, and it decreased gradually to a minimum of 6.90 mm³ at 30 minutes, returning slowly toward baseline but not reaching it by the end of the examination period (volumes at 90 and 180 minutes were 6.95 and 7.08 mm³, respectively). Only a slight decreasing effect of glycerol on the MV occurred in the diabetic patient from 9.88 mm³ at baseline to the nadir, before a rebound effect was observed (MV at 30, 90, and 180 minutes was 9.89, 10.06, and 10.18 mm³, respectively).

**FIGURE 3.** Changes in MV ($\Delta$MV) with the respective regression lines for the healthy (solid regression line) and the diabetic (dashed regression line) groups within the early (0–10 minutes) and the late (15–180 minutes) phases of the examination period. The interaction between time and the presence of diabetes set in shortly after glycerol ingestion. Please note the different scales on each side of the break between 10 and 15 minutes on the abscissa.

**FIGURE 4.** Illustration of the mean changes from baseline in MV for the diabetic and the healthy groups, shown along with the respective SE interval for each time point (error bars). Fitting curves for each group, determined by the mathematical model, are also shown (solid line, healthy subjects; dashed line, diabetic subjects).
subretinal space and the choriocapillaris and/or vitreous compartment, which is concordant with a high osmotic water permeability ($I_p$). Systemic hyperosmolarity induced by mannitol injection increased the fluid absorption rate, but only the local bleb area was examined.

Luan et al.\(^{13}\) used magnetic resonance imaging (MRI) to show that healthy rat retinas subjected to injections with different tonicity exhibited changes in total retinal thickness similarly to an osmometer as well. As the vitreous concentration of contrast (Gd-DTPA) did not increase after the hypo-osmotic load, the authors argued that the subsequent increase in retinal thickness resulted from formation of intracellular edema. However, since regulatory mechanisms react instantly to imbalances of the osmotic homeostasis between the intracellular and extracellular retinal compartments, the interstitial volume innately must have increased as well—at least during the passage of water from the vascular systems. As with the current OCT devices, the MRI scan resolution was insufficient to determine the volume changes on a cellular level.

Similarly, the present OCT technique—and the MRI method—did not allow for the differentiation between the contribution of the inner and outer BRB component to the fluid movements. Nevertheless, the surface area of the retinal capillaries is far greater than that of the RPE, even when the foveal avascular zone is included.\(^{1,4,15}\) Thus, presumably most of the volume changes occurred through the capillary endothelium. This assumption complies with the generally accepted hypothesis that DME primarily originates from the inner retina.\(^{16,17}\)

In another study, we documented that the pharmacokinetics of the osmotic activity of glycerol in the blood are similar in diabetic and healthy persons.\(^{11}\) The difference between the late responses in the two study groups therefore cannot be attributed to differences in the systemic pharmacokinetics of the ingested osmotic substance.

If the osmotic water permeability had been different between the diabetic and healthy subjects, it would have resulted in an altered initial response to the osmotic agent, not in a delayed response, as observed. The only plausible explanation for the latter effect is that glycerol crosses the BRB and thus blunts the osmotic response. This hypothesis was confirmed by our mathematical model, which revealed that BRB permeability to glycerol was more than 10 times higher in the diabetic group than in the healthy group.

Although statistically significant, the volume reductions were clinically insignificant in magnitude. They were transient and followed by an undesirable rebound phenomenon primarily in the diabetic patients. Moreover, unpleasant general side effects as a result of general dehydration occurred.\(^{11}\) Glycerol administration is therefore not recommended for the treatment of DME.

A recent reproducibility OCT study of patients with DME in which Stratus was used (Carl Zeiss Meditec, Inc.) showed that a difference in MV of 0.27 mm\(^3\) (3\%) between two consecutive scans represented the margin of error for a true change.\(^{19}\) In our study, the glycerol-induced variation in MV was less (Figs. 3, 4). Although reliable, the statistically significant differences between the volume changes in the diabetic and the healthy groups were critically dependent on the integration of information from repeated scans of several patients in each group. Thus, a drawback of our method is that the variability of the current OCT-based retinal thickness measurements impedes clinically valuable glycerol permeability estimations in individual patients. With time, the variability of the thickness measurements by spectral domain OCT may be controlled to such an extent that the BRB permeability can be estimated for individual patients. Nevertheless, at present the summed scan rate of their three-dimensional protocols of several seconds still compromises their superiority.

The central (foveal) retinal thickness is usually the preferred OCT parameter in clinical trials, due to the association with the visual acuity. However, a large part of the fovea is devoid of retinal capillaries and hence is without direct proximity to the inner BRB. Since our goal was to study water movements across the BRB, we found the total MV more appropriate, because it reflects a larger retinal area, including the retinal capillaries. Moreover, in patients with DME the variability of Stratus OCT measurements of MV is four times smaller than the variability of central retinal subfield measurements.\(^{18}\)

We agree that the fluid of the retinal cysts is different from the rest of the extracellular fluid in the retina—at least in terms of proximity to the BRB and probably also in respect to higher viscosity. Hence, one could argue that the cyst volume should have been excluded from the analyses. However, this procedure would inevitably have involved bias from the manual definition post hoc of the cyst borders. We therefore found it more appropriate to measure crude retinal volume including the cysts. No obvious volume changes in the cysts were found by simple inspection of the scans, and so the results were not driven by massive in- and outflow of fluid from the cysts.

Second, as retinal cysts are very common among diabetic patients with leaky BRBs and very likely share the same pathogenesis with the other qualitative types of DME, it would be unnatural not to include such patients in the study.

The Stratus fast macular thickness protocol consists of six radial scans, and the retinal map is based on interpolation of the retinal thickness between corresponding A-scans of two neighboring radial lines. With the focal variations in retinal thickness in DME and the decreasing scan density with the distance from the central scan point, this method is inherently inaccurate. Nonetheless, the volume response to a change in the plasma osmolarity is expected to be principally uniform (i.e., independent of the specific scan location on the retina). Therefore, the limitations of interpolation become less important when a large scan area is used.

To our knowledge, the present study is the first to estimate the BRB permeability to glycerol in humans. We found that in patients with DME, the BRB permeability to glycerol was increased 12-fold in the diabetic group over that in the healthy group. This finding agrees with that in a prior fluorometry study, in which the permeability to another marker, fluorescein, was also increased by a factor of 12 in patients with significant DME of various degrees compared with that in healthy control subjects.\(^{7}\)
Ideally, the OCT examinations would have been supplemented by fluorometry on the same day to compare the permeabilities in each study group directly, since the variation within the population of diabetic patients is thought to vary significantly. Fluorometry would have necessitated an additional visit for the participants, which was considered inappropriate, as the glycerol OCT study was quite exhausting for them. From previous fluorometry studies on healthy individuals, the fluorescein permeability has been shown to vary only slightly, between 1 to 2 nm/s in round figures. Although the corresponding variation in permeability is higher in diabetic patients, the quantitative span between the estimates achieved from healthy individuals and patients with clinically significant DME is of equivalent magnitude when compared with the glycerol permeability values. We therefore argue that the glycerol permeabilities obtained by the present OCT method can be compared with previously obtained results on fluorescein permeabilities in healthy and, to some extent, also in diabetic subjects.

As the initial osmotic response to glycerol in the diabetic subjects was almost identical with that in the healthy subjects (Fig. 3), the osmotic response to glycerol was not impaired in the DME patients. Hence, although the present data support the paradigm of BRB breakdown in DME, the barrier functions sufficiently to act as an osmometer in patients with DME. Consequently, the diabetes-induced defects in the BRB are most likely focal by nature, with uneven distribution of the barrier destruction. The typical focal localization of the edemas and the corresponding fluorescein angiograms support this hypothesis. Regarding the BRB solely as a passive membrane passing water and solutes is probably too simplistic.

In both study groups, we found roughly a 30-fold difference in the permeability to fluorescein measured by fluorometry and the permeability to glycerol, as measured by OCT-based MV changes. Since the bulk flow of both glycerol and fluorescein transport across the BRB is probably mediated through specific carrier systems, the difference in the BRB permeability to fluorescein and glycerol is most likely a consequence of different dynamic transport pathways for the two substances, although baseline differences between the two very dissimilar techniques for estimating \( P \) cannot be ruled out. A sodium-mediated glycerol carrier has been found in the rat intestine, and aquaglyceroporins transporting glycerol are located in the skin and adipose tissue. It is therefore reasonable to assume that similar transport systems can be found in the BRB structures, but it has not yet been verified.

The OCT technique for the determination of the BRB permeability to an osmotic agent has advantages when compared with fluorometry, because it is independent of the integrity of the vitreous body and less sensitive to the light attenuation in the optics of the eye, especially in the lens. Traditionally, fluorometry is considered the first choice technique for quantitative assessment of the BRB, but alternative methods have been introduced to find a surrogate marker for BRB permeability. The isotope and Evans blue methods calculate the permeability from the amount of tracer bound to albumin within the retina after a given time. These measurements can be performed only on animals, as they necessitate extraction of the tissue to obtain quantitative analyses.

Trick et al. examined the difference in BRB permeability in diabetic and healthy persons by dynamic contrast-enhanced MRI, which is also reported to be independent of the vitreous integrity. Despite the validity of the procedure, the requirement of an MRI-device limits the clinical implementation (potentially logistically complicated, as it involves another department and is financially costly). In contrast, many specialized ophthalmology clinics now own an OCT instrument, which also has the advantage of easy and fast replication of data to reduce the variability.

Nevertheless, our model was based on an ideal two-compartment system and therefore has limitations. The distribution volume of glycerol affects the estimate of BRB permeability to glycerol. Loss of glycerol to the vitreous causes a diminished osmotic volume response and consequently an underestimation of the permeability.

The model assumes that the entire retinal volume acts as distribution space for glycerol. Although glycerol is probably primarily distributed to the retinal extracellular space, water movements across the cell membranes within the retina will cause rapid osmotic equilibrium and make the entire retinal volume available as distribution space for the osmotic effects of retinal glycerol. It is well documented that the retinal Müller cell membranes contain aquaporin 4, which facilitates rapid redistribution of water within the retina.

The osmotic water permeability \( L_w \) could not be estimated, because the SSD function did not have a minimum for it (Fig. 4). However, the SSD function increased rapidly, with \( L_w < 0.001 \text{ cm/s/Osm} \) (Fig. 4), and we therefore conclude that 0.001 cm/s/Osm is the lowest \( L_w \) compatible with the measured data within the presented model framework.

By coincidence, no patient with type 1 diabetes was included. To our knowledge, no characteristic has yet been identified that discriminates between edemas derived from type 1 or type 2 diabetes. Thus, there is no reason to think that edema in type 1 diabetic patients would have reacted differently to glycerol ingestion. Nevertheless, for scientific integrity we emphasize that the results in the diabetic group are based only on patients with type 2 diabetes.

Diurnal variation in retinal thickness has been documented in several OCT studies on DME. To minimize the effect from the circadian rhythm, the OCT measurements were begun late in the morning, several hours after the participants had arisen from bed. Second, the time curves of the MV approached the baseline value again in both our groups, which contradicts the effect of time of day.

The well-known intraocular pressure-lowering effect of glycerol is expected to be lesser in diabetic patients than in nondiabetic patients, as a consequence of their generally increased BRB permeability. Because of time constraints in the early part of the experimental protocol, we did not monitor intraocular pressure systematically in the present study, but if the reduction in intraocular volume mimics the MV results, one has to consider a shorter therapeutic window and a more severe rebound effect on the intraocular pressure in the diabetic population.

To conclude, the retina responded to peroral glycerol similarly to an osmometer, but a rebound reaction occurred in some of the participants within the 180-minute monitoring period. The present study has introduced a new technique to determine the BRB permeability to osmotic active substances, represented by glycerol, that is independent of the vitreous integrity. In DME, the permeability to glycerol is more than 10 times higher than that in the normal retina, which agrees with previous results on retinal fluorescein permeability. By repeating the present technique on larger molecules such as albumin, it may be possible to determine the significance of the colloid osmotic pressure on the pathophysiology of DME.

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References


