Intravoxel Incoherent Motion Imaging Measurement of Perfusion Changes in the Parotid Gland Provoked by Gustatory Stimulation: A Pilot Study

Anton S. Becker, MD,* Andrei Manoliu, MD, PhD, Moritz C. Wurnig, MD, MSc, and Andreas Boss, MD, PhD

**Purpose:** To demonstrate the feasibility of intravoxel incoherent motion imaging (IVIM) for quantification of perfusion changes in the parotid gland after gustatory stimulation.

**Materials and Methods:** Eight healthy volunteers underwent diffusion-weighted magnetic resonance imaging (MRI) of the neck at 3T with 11 dynamic acquisitions (9 b-values between 0 and 980 s/mm², 2:40 min each). After 5:20 minutes, a lemon-mint-drop was administered orally. Perfusion fraction (Fp), pseudodiffusion (D*), tissue diffusion (Dt) coefficients, and optimal b-value threshold were measured using a multistep variable b-value threshold fitting approach. Dynamic changes in the coefficients between three exemplary timepoints (baseline, after stimulation, after dissolution) were compared using a Mann–Whitney U-test with Bonferroni correction (P < 0.016 significance level).

**Results:** Mean values (95% confidence interval [CI]) for IVIM parameters at baseline were Fp: 0.11 (0.08–0.15), D*: 56.48 mm²/s (39.71–98.27), Dt: 1.01 mm²/s (0.84–1.06), b-value threshold: 30 s/mm² (21.25–105). After stimulation: Fp 0.16 (0.15–0.24; P < 0.01), D*: 93.83 mm²/s (77.98–129.53, P = 0.25), Dt 0.93 mm²/s (0.87–1.08, P = 0.94), b-value threshold 20 s/mm² (13.75–26.25 s/mm², P = 0.10), reflecting the increase in tissue perfusion. After dissolution of the drop: Fp: 0.13 (0.11–0.18, P = 0.38), D*: 101.61 mm²/s (90.68–144.55, P = 0.07), Dt: 0.91 mm²/s (0.85–1.05, P = 0.64), b-value threshold: 15 s/mm² (11.25–40, P = 0.38).

**Conclusion:** The IVIM method allows for simultaneous quantification of changes in perfusion and diffusion effects after gustatory stimulation of the parotid gland.

Impaired secretory function of the salivary glands leading to xerostomia (“dry mouth syndrome”) is a common complication of radiation therapy for head and neck cancer.1 It is also a frequent adverse effect of certain medications, diabetes mellitus, and infectious diseases like HIV.2 Moreover, a number of autoimmune diseases manifest themselves as xerostomia, most notably Sjögren’s syndrome and less commonly systemic lupus erythematosus.3

Saliva acts as an important oral pH buffer and continuously rinses the enamel surface. Its lack causes rampant caries and oral infections.4 Also, speech, taste, and digestion are markedly impaired, leading to a clearly reduced quality of life and contributing to cachexia.

The parotid gland is the largest salivary gland in humans. It is stimulated by vasoactive intestinal peptide, leading to an increase in blood flow.5,6 It stands to reason that impaired blood flow correlates with secretory dysfunction, as suggested by preliminary evidence in Doppler sonography.7,8

To date, only a very limited array of functional imaging modalities for the salivary glands is available. 99mTc-sodium-pertechnetate-scintigraphy has been used to assess salivary gland function.9 It not only implies a poor spatial resolution without depiction of possibly relevant anatomical details, but also carries with it an additional radiation
burden, and may thus be suboptimal for certain populations (e.g., pregnant or pediatric patients). In magnetic resonance imaging (MRI), arterial spin labeling (ASL) has been used to measure perfusion changes in the parotid gland in a small cohort of healthy volunteers. ASL, however, suffers from low signal-to-noise ratio and contamination of the perfusion signal from macroscopic flow in arteries. Moreover, sequences suitable for head-and-neck imaging are not readily available. Recent advances in diffusion-weighted imaging (DWI) have enabled fast image acquisition with acceptable spatial resolution and high signal-to-noise ratio. Consequently, intravoxel incoherent motion imaging (IVIM) has been a research focus in the search for reliable noninvasive tools to detect and quantify tissue perfusion changes.

The underlying principle behind IVIM is the so-called “pseudodiffusion-effect,” which is thought to be a signal decay not due to actual diffusion, but due to fast-moving water molecules in the capillaries.

The purpose of this pilot study was to test the feasibility of the IVIM method in quantifying perfusion changes in the parotid gland of healthy individuals after gustatory stimulation.

Materials and Methods

Study Population

Ethics Committee approval for this study and written informed consent of all individuals was obtained. Imaging was performed on eight healthy volunteers.

MRI Protocol

The measurements were performed on a clinical 3.0T magnetic resonance scanner (Ingenia, Philips Healthcare, Best, Netherlands). All subjects were placed in the supine position and the head and neck were fixed with dedicated pads in a 15-channel head coil. Room temperature was at a constant 21°C. First, conventional morphological T1 and T2-weighted transversal fast spin echo sequences (FSE) as well as a T2-weighted coronal FSE sequence were acquired to 1) determine the size and the precise location of the parotid gland and 2) exclude structural abnormalities that would have influenced DWI measurements, such as a tumor or foreign body. Next, two baseline DWI echo-planar imaging (EPI) sequences before stimulation were acquired (2:40 min each; field of view 240 × 216 mm, voxel size RL × AP: 2 × 2 mm, slice thickness 5 mm, no slice gap, 13–26 slices, reconstructed voxel size: 1.07 mm, TR 2874 msec, TE 64 msec, diffusion time 32 msec, gradient pulse duration 15.8 msec, bandwidth 2245.3 Hz/Px, EPI factor 57, fat suppression by SPIR technique, b-values 0, 10, 20, 40, 80, 160, 320, 640, and 980 s/mm², SENSE factor 2). Afterward, volunteers self-administered a lemon mint herb drop (Ricola, Laufen, Switzerland) and were instructed to let it dissolve in the oral cavity without sucking or other movements, immediately followed by nine identical DW single-shot sequences repeated over a total of 25 minutes. The temporal scheme of the dynamic DWI acquisition is outlined in Fig. 1. In two volunteers, a lemon mint herb drop was given on a separate occasion with the same instructions, and the time until dissolution was taken.

Postprocessing Algorithm

A proprietary 3D motion correction algorithm (“diffusion registration package”) integrated in the MR-workstation was applied to all images. The IVIM analyses were performed using in-house MatLab routines (r2015b, MathWorks, Natick, MA). A polygonal region of interest (ROI) was placed in the middle of the corpus of the right parotid gland. This was done to account for any small movements potentially not compensated by the aforementioned algorithm, and to avoid field inhomogeneities and susceptibility artifacts in proximity to the mandible. ROI analysis was performed by an investigator blinded to the details of the scanning/stimulation protocol. The ROIs were placed in the b0-slice depicting the largest part of the corpus of the parotid gland and copied to the corresponding slices of the same and remaining b-values at all timepoints, yielding 11 decay curves each consisting of nine b-values on the abscissa and the signal intensity on the ordinate.

\[
\frac{S_b}{S_0} = (1 - F_p)\exp(-bD_t) + F_p\exp(-bD^*)
\] (1)

The biexponential Eq. 1 describes the signal decay in DWI according to the IVIM paradigm. \(S_b\) represents the signal at the different b-values, \(S_0\) the signal at \(b = 0\) mm²/s, \(D_t\) the tissue diffusion coefficient, \(D^*\) the pseudodiffusion coefficient, and \(F_p\) the relative perfusion fraction. The latter two will superimpose the signal decay caused by the tissue diffusion at low b-values (typically assumed <150 mm²/s²).

IVIM analysis for each timepoint was performed on a single slice using a multistep variable b-value threshold fitting approach as follows:

1) \(D\) is calculated from the highest \(n\) b-values under the assumption that perfusion effects do not significantly contribute to the signal decay at high b-values. Initially, \(n\) is equal to the number of b-values. \(D\) is calculated using a first-order polynomial fit to the log-transformed signal intensities:

\[
\log S_b = -D \times b + \log S_0
\] (2)

2) The perfusion fraction \(F_p\) is subsequently calculated from the measured signal intensity at \(b = 0\) (\(S_0\)) and the derived \(S_0'\):
Substituting D and \(F_p\) with these values, \(D^*\) is computed for all b-values by fitting the signal intensities to Eq. 1 using a nonlinear least-squares algorithm based on the Levenberg-Marquardt technique. The MatLab function used for this task (“lscurvefit”) also provides the sum of the squared residuals to the fit.

Steps 1–3 are repeated without the next lower b-value in steps 1 and 2 to determine the initial D and \(F_p\). Therefore, the algorithm will loop \(n\)-1 times through the data with \(n\) equal to the number of b-values in the protocol. In the end, only the two highest b-values remain, as for the highest b-value alone no polynomial fit can be calculated.

The optimal b-value threshold and corresponding IVIM-parameters can now be determined by choosing the b-value threshold with the lowest number of squared residuals from step 3, meaning the best fit to the actually measured signal intensities.

Statistical Analysis
Statistical tests were performed in R v. 3.2.3. Differences in IVIM parameters between sequences 1, 3, and 11 (henceforth termed before, during, and after stimulation) were tested with a Wilcoxon test for paired observations (“Mann-Whitney-test”). \(P < 0.05\) was considered statistically significant; Bonferroni correction for multiple comparisons was applied which yielded a \(P < 0.016\) at the chosen 5% significance level. Plots were created with ggplot2. For the graphical evaluation, the product of \(F_p\) and \(D^*\) was used, which has been proposed as a more robust measure of tissue-specific perfusion than the parameters alone.

Results

Study Population
The final study population consisted of five male and three female volunteers. Mean age was 30 years (range 21–50 years). None of the volunteers had a history of or complaints consistent with xerostomia.

Image Acquisition and Postprocessing
Time to lemon mint herb drop dissolution = stimulation time was 21–25 minutes. All measurements were completed without significant distortions or artifacts (sample slices depicted in Fig. 2). No large movements between the different timepoints were noted. Typical morphological FSE
images and a representative DWI image acquired at $b = 0 \text{mm}^2/\text{s}$ are depicted in Fig. 1. Postprocessing was successfully applied to all images. A typical fitting process of two curves at different timepoints in the same volunteer is depicted in Fig. 3a, with the best fit drawn in bold ($b$-threshold $= 20 \text{ s/mm}^2$, green). In Fig. 3b, example curves of one volunteer are shown at three different timepoints before, during, and after gustatory stimulation.

**Quantitative Values Before, During, and After Gustatory Stimulation**

Median values (and bootstrapped 95% confidence intervals) before the gustatory stimulation were $F_p: 0.11 \ (0.08-0.15)$, $D^*: 56.48 \text{mm}^2/\text{s} \ (39.71-98.27)$, $D_t: 1.01 \text{mm}^2/\text{s} \ (0.84-1.06)$, $b$-value threshold: $30 \text{ s/mm}^2 \ (21.25-105)$. In the sequence during stimulation, $F_p$ rose to $0.16 \ (0.15-0.24; P < 0.01)$, a 45% increase, while $D^*$ showed only a nonsignificant trend with $93.83 \text{mm}^2/\text{s} \ (77.98-129.53, P = 0.25)$; $D_t$ and the $b$-value threshold remained relatively stable at $0.93 \text{mm}^2/\text{s} \ (0.87-1.08, P = 0.94)$ and $20 \text{ s/mm}^2 \ (13.75-26.25, P = 0.10)$. Towards the end of the examination, 15 minutes after beginning of the stimulation, $F_p$ started to fall again before almost reaching the initial level with $0.13 \ (0.11-0.18, P = 0.38 \text{ compared to baseline})$ in the last sequence; with the remaining coefficients similar to baseline as well: $D^*: 101.61 \text{mm}^2/\text{s} \ (90.68-144.55, P = 0.07)$, $D_t: 0.91 \text{mm}^2/\text{s} \ (0.85-1.05, P = 0.64)$, $b$-value threshold $15 \text{ s/mm}^2 \ (11.25-40, P = 0.38)$. These comparisons are summarized in the boxplot of Fig. 4. A graphical representation of the whole time course of the median values with respective confidence intervals is shown in Fig. 5. An illustration of the residuals for all $b$-value thresholds at three different timepoints, with the optimal thresholds at the local minima, in one volunteer is given in Supplemental Figure 1. The detailed measurements for each volunteer are provided in Table 1.

**Discussion**

In this IVIM study, we quantitatively and noninvasively measured the physiological blood flow changes in the healthy parotid gland after gustatory stimulation. We were able to show that the blood-flow-dependent parameters $D^*$ and $F_p$ can reliably be computed from conventional DWI sequences at different $b$-values in the parotid gland. Changes in the perfusion dependent parameters could be observed using a gustatory stimulation for activation of the salivary gland.

In the clinical routine, salivary gland scintigraphy with $99\text{mTc}$-sodium-pertechnetate is used for the evaluation of salivary gland function with parameters such as uptake ratio, maximum accumulation, and maximum secretion. However, this modality has several drawbacks over MRI, as mentioned above.

MRI offers several options to measure perfusion changes without application of contrast media. In the past, arterial spin labeling (ASL) with a flow-sensitive alternating inversion recovery (FAIR) spin-preparation combined with a true fast imaging in steady-precession data readout (True-FISP) sequence has been used to assess tissue perfusion in the parotid gland after gustatory stimulation. It is a single-slice acquisition technique providing only information on one representative slice of the parotid gland, which requires a difficult planning process before the image acquisition. DWI provides a complete coverage of a 3D volume, which is typically used for purposes of ischemia or tumor diagnosis. In the past, DWI of the head and neck area was...
hampered by susceptibility artifacts due to bone–soft tissue–air interfaces. In recent years, intensive research efforts in DWI have lead to robust DWI sequences with sufficient spatial resolution.21–24 In the brain, the product of pseudo-diffusion coefficient $D^*$ and perfusion fraction $F_p$ have been shown to correlate with the tissue perfusion measured after bolus contrast injection.14,25 Although a direct bilinear fitting approach with a fixed $b$-value threshold may be used to determine the IVIM parameters, we used a multistep variable $b$-value threshold fitting approach as described above. The multistep approach dynamically adapts to changes in the threshold between diffusion and perfusion effect and thus delivers more consistent results, because an arbitrarily chosen fixed $b$-value threshold leads to changes in all derived parameters $D^*$, $F_p$, and $D_t$.26

A previous study, Thoeny et al detected changes in the apparent diffusion coefficient (ADC) in DWI measurements in salivary glands after stimulation;27 Kato et al found that this ADC difference is smaller in patients with xerostomia.28 We suspect that these small but significant changes in ADC may have actually been pseudodiffusion and not true diffusion, since the authors used a monoexponential model to calculate the ADC. Acquiring multiple low $b$-values and applying the biexponential IVIM analysis introduces the possibility to separate this pseudodiffusion from true diffusion-related signal decay.29 We postulate that if performed in patients with xerostomia, IVIM would show a reduced change in $F_p$ and $D^*$ after stimulation with a stable $D_t$ ($\sim$ADC).

In large organs such as the brain or liver, the contribution of macroscopic vessels, which carry blood to a distant part of the organ but do not directly contribute to the local blood supply of the tissue in the ROI, has to be considered as a contamination source of the perfusion signal.30 In the parotid gland, a very small encapsulated organ, this effect seems negligible and the perfusion fraction probably represents a surrogate marker essentially determined by the local capillary blood flow. However, several effects might result in additional contributions to the IVIM parameters: First, the blood vessels of the salivary glands not only supply the glandular cells, but also form a dense vascular mesh around the ducts, where a relatively high volume of serous saliva is produced in a short time, possibly through active transepithelial ion transport and coupled water transudation.31 Second, the flow of the saliva in the excretory ducts may contribute to the perfusion-related IVIM parameters. Further examination of this phenomenon would require more complex mathematical models with three compartments, as have been proposed for the liver with its dual blood supply.32 Third, the presence of small arteriovenous anastomoses in
the parotid gland has been discussed, which may result in micro-shunts bypassing the capillary bed. This would mean that the calculated “perfusion fraction” \( F_p \) does not completely represent the actual “tissue perfusion” (meaning transportation of oxygen and nutrients through blood flow).

The activity and thus the microcirculation of the parotid glands is influenced by neuropeptides, which are mainly under parasympathetic control. In our subjects we observed a significant increase of \( F_p \) immediately after the start of the gustatory stimulation, which was maintained during the whole period while the lemon mint herb drop dissolved in the oral cavity, declining slowly after dissolution. The concurrent saliva secretion is a result of degranulation of the glandulocytes in the parotid acini. In rats, this process has been demonstrated to leading to a reduction of free water in the extracellular space. From our stable \( D_t \) and b-value threshold we conclude that this effect is too small to be reliably detectable in a small number of volunteers. The relative change of the \( F_p \) coefficient of 41.5% in our group is in concordance with previously reported data on parotid perfusion changes after gustatory stimulation using ASL.

Our study has several limitations. 1) Due to the need for acquisition of multiple b-values, a compromise between number of b-values and temporal resolution had to be made. With the now known optimal b-value threshold, fewer b-values could potentially be used in future studies, improving the temporal resolution. 2) Our volunteers were fairly young and are not a representative sample of the usual hospital population, which consists mainly of elderly patients, especially in the context of oncology/postirradiation syndrome. Since saliva secretion is not age-dependent, however, we do not think that including more seasoned volunteers would have altered our results. 3) Our study design only included a small number of healthy individuals, and no patients with xerostomia. 4) Some intraindividual variability was still observed, and it remains to be shown whether the technique is stable enough to detect pathological changes in individual patients. 5) In this proof-of-concept study, although a complete stack of DWI images was available, a single slice in the center of the parotid gland was evaluated due to the standardization of the evaluation process.
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**IVIM Parameters for Each Volunteer at Every Timepoint**

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- **Dt [10^{-3} \, \text{mm}^2/\text{s}]**
- **Fp**
- **D* [10^{-3} \, \text{mm}^2/\text{s}]**
- **b-value Threshold [\text{s/mm}^2]**

**TABLE 1.**
In conclusion, IVIM measurement in the parotid glands is a feasible noninvasive functional imaging option to measure blood flow changes in the glands due to gustatory stimulation. This quantitative technique could possibly be used to diagnose and grade xerostomia in patients suffering from Sjögren or postirradiation syndrome.

Acknowledgments
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References


