Scientific Research Publishing

^{31}P MR Spectroscopy with 3D Chemical-Shift Imaging Detects Changes in Levels of Phosphorus Metabolites Due to Saliva Secretion in Human Parotid Glands

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Abstract

Objectives: To measure phosphorus metabolites in human parotid glands by ^{31}P-MRS using three-dimensional chemical-shift imaging (3D-CSI), and ascertain whether this method can capture changes in adenosine triphosphate (ATP) and phosphocreatine (PCr) levels due to saliva secretion. **Study Design:** The parotid glands of 20 volunteers were assessed by ^{31}P-MRS using 3D-CSI on 3T MRI. After obtaining a first (baseline) measurement, the participants took vitamin-C tablets and measurements were obtained twice more, in a continuous manner. The peak area ratios of PCr and β-ATP were evaluated. **Results:** A high proportion of PCr (0.26 ± 0.08) and ATP (α: 0.16 ± 0.06; β: 0.27 ± 0.06; γ: 0.21 ± 0.03) was noted at baseline. A significant decrease in β-ATP was observed between baseline ("pre"; 0.27 ± 0.06) and the first scan after vitamin-C stimulation ("post-1"; 0.19 ± 0.07, $p < 0.05$). There was a near-significant decrease in PCr between pre (0.26 ± 0.08) and post-1 (0.23 ± 0.06, $p = 0.074$). **Conclusions:** ^{31}P-MRS with 3D-CSI can assess the major phosphorus metabolites in human parotid glands and capture changes in their levels due to saliva secretion. This technique is simple, non-invasive, and provides new information regarding saliva secretion.

Keywords

^{31}P-MRS, 3D-CSI, Parotid Gland

1. Introduction

The two parotid glands are the largest of the human salivary glands. They secrete

saliva via the parotid duct into the oral cavity to facilitate mastication and swallowing. Resting (unstimulated) salivary production occurs mainly in the submandibular, sublingual, and numerous small oral salivary glands. In contrast, the parotid glands are largely responsible for stimulated salivary production (60% - 70% of the total) [1]. Therefore, the change of function that occurs in the parotid glands at meal time is very important for appropriate dietary intake.

Magnetic resonance spectroscopy (MRS) classifies the magnetic-resonance signals of metabolites into frequencies using the difference in the resonance frequency of nuclei depending on the structure and environment of metabolites. MRS can be used to identify information such as the type of metabolite, its concentration, and the relaxation time. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) is a non-invasive technique that provides useful information regarding the metabolic and phosphoenergetic status of human tissue, in both physiological and pathological conditions [2] [3]. In addition to the information contained within static spectra, dynamic ^{31}P-MRS has been used to assess changes in the levels of phosphorus metabolites [4] [5] [6]. Furthermore, three-dimensional chemical-shift imaging (3D-CSI) enables metabolic information and detailed information on position to be obtained [7] [8]. For the past three decades, ^{31}P-MRS studies have been conducted using human tissues (e.g., muscle, heart, liver, brain); however, most studies on the salivary glands have been carried out in vitro in animal experiments [9]-[14], and only one study of static spectra in human parotid glands has been published [15].

Numerous previous studies have reported phosphocreatine (PCr) and adenosine triphosphate (ATP) levels measured by MRS in the excised glands of animals and in human parotid glands [9]-[15]. In addition, creatine phosphokinase has been purified from the submandibular glands of rats [16]. These findings indicate the existence of the Lohmann reaction in the salivary gland, in which adenosine triphosphate (ATP) is regenerated by transphosphorylation from PCr to adenosine diphosphate (ADP). It has been shown in MRS experiments using excised animal glands that ATP and PCr are consumed by salivary secretion [9]-[14], but reports on physiological changes in human salivary glands in vivo are lacking.

We assessed phosphorus metabolites in human parotid glands by ^{31}P-MRS using 3D-CSI on 3T MRI. We evaluated whether this method could be used to capture changes in the levels of high-energy phosphorus metabolites (ATP and PCr) due to saliva secretion.

2. Materials and Methods

2.1. Ethical Approval of the Study Protocol

The study was conducted in accordance with the ethical standards set by the World Medical Association (Declaration of Helsinki). Approval for the study protocol was obtained from the Institutional Review Board/Ethics Committee of the Kyoto University Graduate School of Medicine (R1382) on 22 February

2018. Written informed consent was obtained from each participant prior to MRI.

2.2. Participants

Between March and July 2018, 20 healthy adult volunteers (12 men and 8 women; age range, 25 - 64 years; mean age, 36.8 years) were recruited through advertisements. The exclusion criteria were a history of salivary-gland disorders, communication difficulties, or contraindications to MRI (e.g., claustrophobia, metallic implants).

2.3. MRS Examinations

MRS data were acquired on 3T MRI (Magnetom Skyra; Siemens Healthcare, Erlangen, Germany), using a $^{31}P/^{1}H$ loop transmit-receive radiofrequency coil (Takashima Seisakusho, Tokyo, Japan) with a diameter of 120 mm. Participants were placed in the right-lateral position in the magnet with the coil positioned over the lateral aspect of the right parotid gland. ^{1}H T1-weighted ultrafast gradient-echo images (TR = 4.00 ms, TE = 0.99 ms, FoV = 400 mm, base resolution = 256, slice thickness = 4.5 mm, acquisition time = 48 s) were obtained covering the right parotid gland, and these were used for voxel placement in ^{31}P spectroscopic imaging. 3D ^{31}P spectroscopic images were obtained with the following parameters: TR = 1000 ms, TE = 2.3 ms, bandwidth = 3000 Hz, FoV = 200 × 200 × 200 mm, matrix = 9 × 9 × 8, and flip angle = 90˚. The matrix size was interpolated to 16 × 16 × 16, resulting in an interpolated voxel size of 2.2 × 2.2 × 2.5 cm, or 12.3 cm³. Weighted averaging (12 averages) was applied, resulting in a total acquisition time of 10 min, 32 s. ^{31}P MR spectra were acquired three times in all participants. As a baseline, the first measurement (pre) was obtained after 3 h of fasting. The subjects were then asked to take tablets containing 244 mg of vitamin C, and measurements were obtained twice more, in a continuous manner (post-1 and post-2).

2.4. Analyses of ³¹P Data

Analyses were undertaken by a single radiologist who had 7 years of experience. On the T1-weighted ultrafast gradient-echo images, a voxel in a homogeneous area (to avoid blood vessels) of the right parotid gland was chosen for evaluation. Spectra were analyzed with Syngo software (Syngo Spectroscopy Evaluation; Siemens Medical Systems). The intensities of phosphocholine, phosphoethanolamine, inorganic phosphate, glycerophosphoethanolamine, glycerophosphocholine, PCr, and ATP were fitted in the frequency domain using the least-squares method. Results were expressed as the ratio of the peak area to the total phosphorus signal.

2.5. Statistical Analyses

Results are expressed as the mean ± standard deviation (SD). The β-ATP peak

was used to estimate the ATP level because this peak does not contain adenosine monophosphate or ADP. Significant changes in the time-course of PCr and β-ATP were evaluated with one-way analysis of variance. Overall significant differences during the three-time points were further tested using the paired *t*-test with Bonferroni's correction. Statistical tests were two-sided, and p < 0.05 was considered significant. Statistical analyses were performed using R v3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

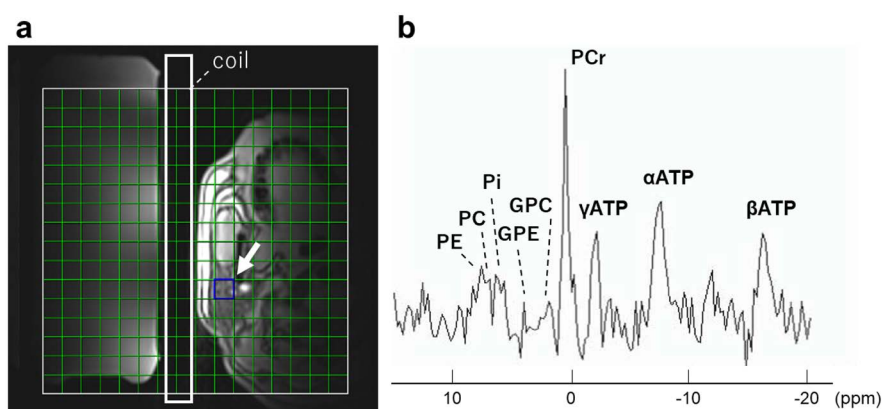Nine peaks were depicted in the ^{31}P-MRS spectrum (**Figure 1**). **Figure 2** shows

Figure 1. Axial MR image (a) of a parotid gland showing the selected voxel (arrow), and corresponding in vivo spectrum (b) acquired by 3D-CSI in 10 min, 32 s. The assigned metabolite resonances are as follows: PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphate; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PCr, phosphocreatine; ATP, adenosine triphosphate.
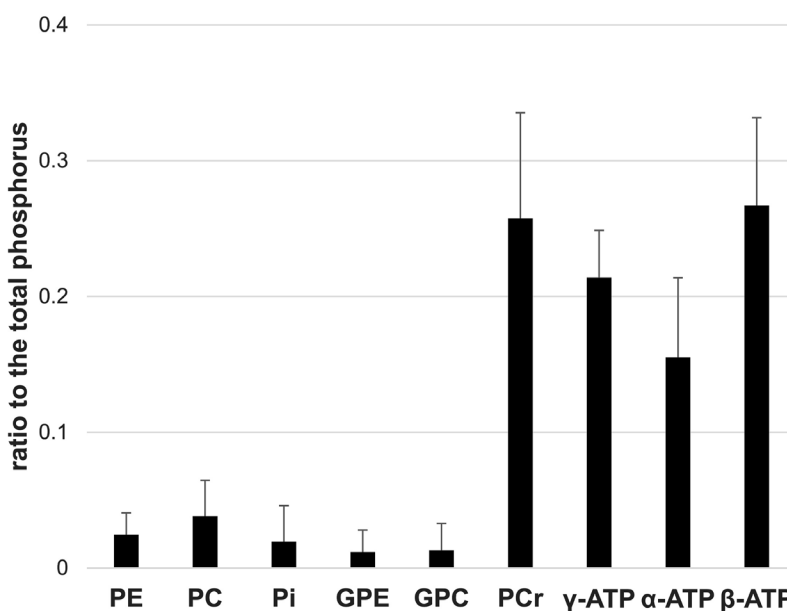

Figure 2. Levels of major phosphorus metabolites (mean ± SD) in the parotid glands of 20 healthy volunteers.

the mean ± SD of the ratio of each substance to the total phosphorus signal before vitamin-C stimulation in the 20 participants. The highest ratios were found for the high-energy phosphorus metabolites PCr (0.26 ± 0.08) and ATP (α: 0.16 ± 0.06; β: 0.27 ± 0.06; γ: 0.21 ± 0.03). The signal-to-noise ratio of β-ATP and PCr was sufficient for detection and analysis (β-ATP: 5.05 ± 1.76; PCr: 6.66 ± 2.77).

Figure 3 shows β-ATP and PCr levels before and after vitamin-C stimulation. A significant change was observed in the time-course of β-ATP ($p < 0.05$). Multiple comparisons revealed a significant decrease between pre (0.27 ± 0.06) and post-1 (0.19 ± 0.07, $p < 0.05$), and there was a nearly significant increase between post-1 (0.19 ± 0.07) and post-2 (0.26 ± 0.08, $p = 0.08$). There was a significant change in the time-course of PCr ($p < 0.05$) and a nearly significant decrease
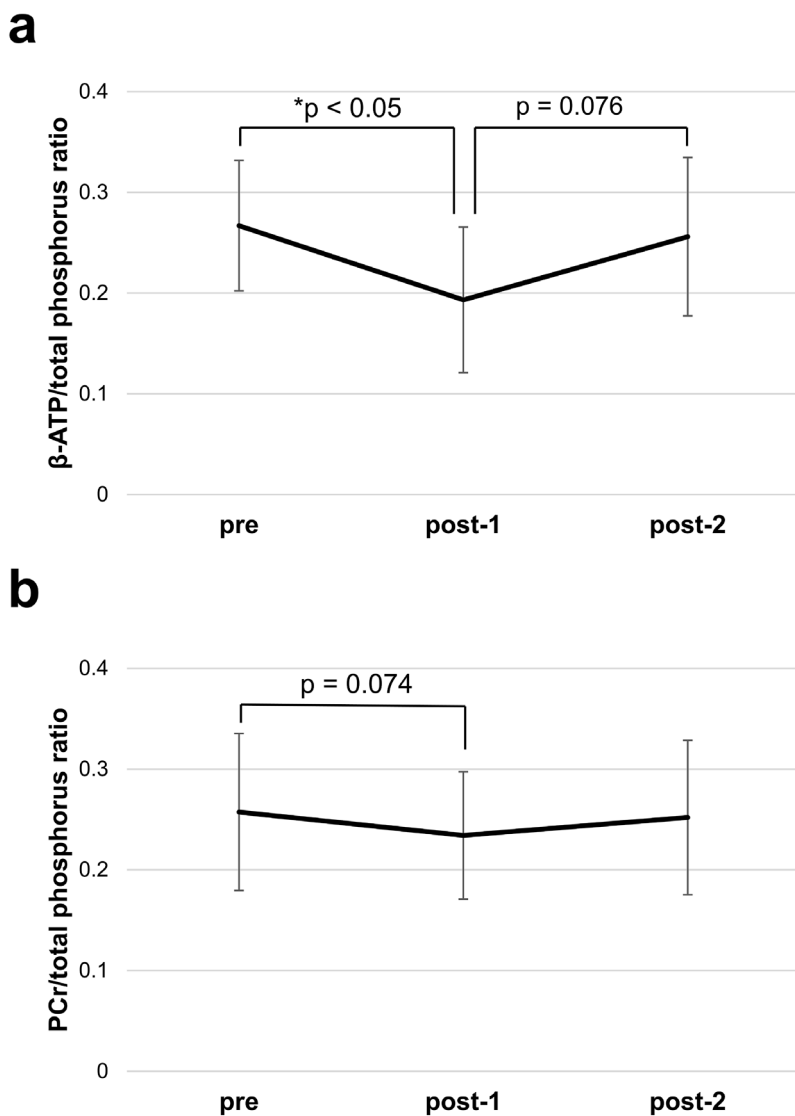

Figure 3. Levels of phosphorus metabolites (mean ± SD) at baseline and after vitamin-C stimulation. Ratio of the peak area to the total phosphorus signal for (a) β-adenosine triphosphate (β-ATP) and (b) peak area ratio to the total phosphorus signal for phosphocreatine (PCr). *Denotes significant differences between groups at $p < 0.05$.

between pre (0.26 ± 0.08) and post-1 (0.23 ± 0.06, p = 0.07). There was a slight increase in the mean ± SD between post-1 (0.23 ± 0.06) and post-2 (0.25 ± 0.08), but this change was not significant (p = 1.00).

4. Discussion

Saliva secretion requires the coordinated activity of multiple water transporters, ion transporters, and ion-channel proteins, and phosphorus metabolism plays an important part. In the salivary glands, ATP is consumed by Na^+/K^+ ATPase, which is believed to establish a Na^+ gradient across cell membranes to create the driving force for Na^+-K^+-Cl^- cotransport systems. Cl^- is then released to the lumen through calcium-activated apical Cl^- channels. Following the movement of Cl^-, Na^+, and water into the lumen, saliva is secreted [17] [18] [19].

The aim of our study was to assess phosphorus metabolites in human parotid glands using ^{31}P-MRS with 3D-CSI on 3T MRI, and additionally evaluate whether this technique could capture changes in phosphorus metabolites due to saliva secretion.

We detected a high proportion of the high-energy phosphorus metabolites PCr and ATP. ATP levels decreased significantly after vitamin-C stimulation, and PCr levels decreased to near-significant levels. These data suggest that, in vivo, ^{31}P-MRS with 3D-CSI can be used to detect major phosphorus metabolites in the human parotid gland within a reasonable scan time, and to reveal the consumption of high-energy phosphorus metabolites due to saliva secretion.

The spectra showed obvious peaks due to the high-energy phosphorus metabolites ATP and PCr. Our results are in accordance with reports from human and in vitro animal experiments [9]-[15]. Only one of these was a clinical study (15), which was performed using 1.5T MRI, compared with 3T MRI in the present study. In theory, a higher field strength should be advantageous for MRS because of its greater sensitivity and chemical-shift resolution, and shorter acquisition time [20] [21] [22]. The study that used 1.5T MRI [15] employed the surface-coil localization method and achieved only a static spectrum, with an acquisition time of 35 min. Furthermore, no detailed information on position was obtained. However, in the present 3T MRI study, even though we employed 3D-CSI and obtained high spatial resolution, we could still achieve a shorter acquisition time (10 min, 32 s per scan) and continuous imaging. Also, we considered that the use of 3D-CSI would enable us to avoid the partial-volume effect and to reduce signal contamination, particularly that from muscles (which are rich in ATP and PCr).

A high signal intensity of PCr has been reported for the salivary glands, skeletal muscle, heart, and brain [4] [20] [21]. The Lohmann reaction (a reversible mechanism for maintaining the ATP concentration) exists in these organs, producing ATP by transferring high-energy phosphate groups from PCr to ADP. PCr is produced by the creatine kinase present in mitochondria, and it moves into cytoplasm with a high diffusion coefficient. On reaching the target ATPase,

the high-energy phosphate group is transferred from PCr to ADP to produce ATP [22]. It is believed that PCr is abundant in organs that have a high demand for rapid energy. Our results indicate that physiological changes in the salivary glands require rapid liberation of energy.

We detected a decrease in the levels of high-energy phosphorus metabolites during saliva secretion upon vitamin-C stimulation. ^{31}P-MRS has been used for three decades in studies of physiological changes in muscles, brain, and liver. However, studies of the salivary glands have been performed only with excised and perfused animal glands, and research on saliva production in humans is lacking. In animal experiments, reductions in PCr levels were more apparent than those of ATP [9]-[14]. In the present study, a significant decrease in ATP levels was observed after saliva secretion, whereas the decrease in PCr levels was nearly significant. Further work is needed to explore the reason for this result.

Methods for assessing the salivary glands include the gum method and the Saxon test to measure saliva secretion on stimulation, the spitting method to measure resting saliva secretion, and scintigraphy to evaluate function [23] [24]. Phosphorus metabolites can provide unique information other than the amount of saliva. It has been reported that static spectra of the salivary glands of patients with Sjögren's syndrome reveal low levels of ATP and PCr [15]; further information could be obtained using our method. In addition, by obtaining information regarding metabolism and position simultaneously with 3D-CSI, it may be possible to evaluate the function of each salivary gland.

Our study had three main limitations. First, we included only healthy volunteers in the study cohort. In future studies, it will be necessary to compare the results in healthy volunteers with those of patients with failure of salivary secretion. Second, we assessed the results in terms of the ratios of levels of metabolites. Although relative quantification is a convenient and useful way to capture trends, it is necessary to evaluate the absolute concentration to obtain more accurate and unambiguous results. Third, the resonances of phosphocholine, phosphoethanolamine, inorganic phosphate, glycerophosphoethanolamine, and glycerophosphocholine were lower than those stated previously [9]-[15] because we used a shorter TR to achieve a tolerable examination time. Nevertheless, this was not a serious problem in the present study because the main purpose was to evaluate changes in levels of the high-energy phosphorus metabolites ATP and PCr.

5. Conclusion

In conclusion, we demonstrated that 31P-MRS with 3D-CSI can be used to evaluate levels of the major phosphorus metabolites in human parotid glands, and to capture the change in levels of high-energy phosphate groups due to salivary secretion. Acquisition time was 10 min, 32 s per image, and it was possible to acquire images continuously. The examination was not burdensome for the study participants. Our technique could be applied in much larger studies to

evaluate the pathogenesis of saliva-secretion failure (e.g., Sjögren's syndrome and radiation-induced salivary gland dysfunction).

Acknowledgements

Funding

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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