

ORIGINAL**Reproducibility and Variability of Quantitative Cerebral Blood Flow Measured by Multi-delay 3D Arterial Spin Labeling According to Sex and Menstrual Cycle**

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Abstract : Purpose : To determine the reproducibility of corrected quantitative cerebral blood flow (qCBF) through measurement of transit flow time using multi-delay three-dimensional pseudo-continuous arterial spin labeling (pCASL) in healthy men and women and to evaluate the differences in qCBF between not only men and women, but also the follicular and luteal phases of the women's menstrual cycle. **Methods :** The participants were 16 healthy volunteers (8 men and 8 women ; mean age, 25.3 years). Two MRI were conducted for all participants ; female participants were conducted in the follicular and luteal phases. The reproducibility of qCBF values was evaluated by the intraclass correlation coefficient (ICC) and differences between the two groups were estimated by voxel-based morphometry (VBM) analysis. **Results :** The qCBF values were lower in men than in women, and those in females were significantly different between the follicular and luteal phases ($P < 0.05$). In VBM analysis, the qCBF values of the lower frontal lobes were significantly higher in women than in men ($P < 0.05$). The qCBF values of the frontal pole were significantly higher in the follicular phase than in the luteal phase ($P < 0.01$). **Conclusion :** Multi-delay pCASL can reveal physiological and sex differences in cerebral perfusion. *J. Med. Invest.* 67: 321-327, August, 2020

Keywords : neuroimaging, arterial spin labeling (ASL), cerebral blood flow (CBF), 3DSRT, gender difference

INTRODUCTION

Arterial spin labeling (ASL) perfusion imaging without contrast medium is widely used in the clinical evaluation of various patients. However, it has also been reported that the transit flow time greatly influences the signal intensity and that the duration of the post-labeling delay (PLD) alters the quantitative cerebral blood flow (qCBF) measured by ASL (1-3). Although pseudo-continuous ASL (pCASL) is generally less affected by transit flow time than pulsed ASL (4), several studies have recommended the use of multiple PLD times to correct the qCBF determined by pCASL (3, 5).

In clinical practice, sufficient reproducibility of the qCBF derived from pCASL is important because ASL is conducted to differentiate pathological from physiological status and to evaluate longitudinal changes in pathological severity or therapeutic effects. Several studies have evaluated the reproducibility of ASL using a single PLD time (6-8). However, as far as we know, no study has evaluated the reproducibility of corrected qCBF values using multi-delay pCASL.

On the other hand, some of the studies reported that qCBF was higher in women than in men (9,10), indicating a sex difference in qCBF. Furthermore, qCBF may be influenced by the hormonal balance of the menstrual cycle in women (11). Hence, we hypothesized that differences in sex and menstrual cycle phase might cause variability in qCBF.

Accordingly, the purposes of this study were to determine the

reproducibility of corrected qCBF values through measurement of transit flow time using multi-delay three-dimensional pseudo-continuous arterial spin labeling (pCASL) in healthy men and women and to evaluate the differences in qCBF between not only men and women, but also the follicular and luteal phases of the women's menstrual cycle.

MATERIALS AND METHODS*Participants*

Sixteen healthy volunteers (men, n = 8 ; women, n = 8 ; mean age, 25.3 years ; range, 20-28 years) participated in this MRI study after providing written informed consent.

The volunteers underwent two MRI scans at almost a fixed time in the afternoon to avoid the effect of the circadian rhythm of sex hormones ; the second scans were performed 2-3 weeks after the initial MRI study. The participants were instructed to relax and keep their eyes closed during the scan, but not to sleep. The MRI scans of the female participants were conducted during the follicular and luteal phases after determining each participant's menstrual cycle. All women participants have regular menstrual cycle. All volunteers were confirmed by interview that they had no history of oral administration or medical history. This research is approved by the ethics committee of the Tokushima University.

MRI data acquisition

The experiments were performed using a 3.0 T MRI scanner (Discovery 750 ; GE Healthcare, Milwaukee, WI). The body coil was used for RF transmission and an 8-channel head-neck coil was used as the receiver.

First, high-resolution sagittal T1 images for normalization and template creation were collected using inversion recovery (IR)

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three-dimensional (3D) Spoiled Gradient Recalled (SPGR) with the following parameters: repetition time (TR), 8.57 ms; echo time (TE), 3.53 ms; preparation time, 400 ms; flip angle (FA), 15°; bandwidth, 41.67 Hz/pixel; voxel size, 1.6 × 1.6 × 1.6 mm³; field of view (FOV), 220 × 220 mm²; and number of slices, 176. At the same time, 3D SPGR was used to check for structural abnormalities in the brain.

We performed 3D pCASL imaging with three PLD times with the following parameters according to the previous reports (12,13): FSE spiral readout; TR, 6015 ms; TE, 11.2 ms; FOV, 240 × 240 mm²; slice thickness, 4 mm; data sampling, 6 spirals × 600 sampling points; image matrix, 128 × 128; number of slices, 36; three different PLD times of 1.00 s, 1.57 s, and 2.46 s; and effective label durations of 0.57 s, 0.89 s, and 2.04 s, respectively. The shortest delay time was chosen to be 1.00 s to allow sufficient time for the T₂-preparation module to be completed. The longest delay time was 2.460 s to obtain a compromise between the range of coverage and signal-to-noise. The other delay times were chosen to have approximately equal intervals. By setting the labeling duration for exponential in consideration of the signal degradation due to the difference in PLD, the SNR of the image in each PLD is nearly equal, and the SNR of the corrected image is kept high. The order of the scans was pseudo-randomized across the participants according to previous work (12).

Foam padding was used to stabilize the head and minimize movement. The blood labeling position was chosen to be 20 mm below the imaging slab. The imaging position ensured that the patient's head was as straight as possible and that the bottom of the imaging volume was near the bottom of the cerebellum.

Data analysis

Quantitative evaluation

qCBF maps were generated using the software on the GE scanner console computer with the method proposed by Wang *et al.* (14). qCBF was calculated using the following equation:

$$CBF = C \cdot \frac{\lambda(SI^{lab} - SI^{non-lab}) \cdot PLD/e^{t_1 b}}{2\alpha T_{1b} \cdot (1 - e^{T_{1b} b})} \text{ (mL/100 g/min)},$$

where C is 6000/SI^{PD}, α is the labeling efficiency (0.85), λ is the brain-blood partition coefficient in mL/g (blood-tissue, 0.9), SI^{PD} is the signal intensity of a proton density-weighted image, SI^{lab} and SI^{non-lab} are the time-averaged signal intensities in the labeled and non-labeled images, respectively, T_{1b} is the longitudinal relaxation time of blood, e is the base of the natural logarithm (Napier's number), τ is the label duration, and PLD is the post-labeling delay.

The transit flow time maps were generated by three different PLD times and qCBF maps after correction by the transit flow times according to the formula of Dai W. *et al.* (12,13) and the reproducibility of the technique were evaluated.

Image analysis

Image processing was performed using Statistical Parametric Mapping (SPM) software (SPM12; University College London, London, UK) and MATLAB R2019a scripts (The MathWorks, Inc., Natick, MA). Preprocessing involved the steps realignment (the reference volume was the first obtained echo planar imaging (EPI) volume) and unwrapping, normalization of the anatomy using a template image provided by the Montreal Neurological Institute (MNI), resampling with a voxel size of 4 × 4 × 4 mm, and smoothing (8 mm full width at half maximum (FWHM)).

Three-dimensional stereotaxic region-of-interest template (3DSRT) software (FUJIFILM Toyama Chemical, Tokyo, Japan), developed by Takeuchi *et al.* (15), was used to obtain regional values. 3DSRT is an automated region-of-interest (ROI) analysis software that deforms a normal brain template and establishes identical ROIs on anatomically standardized brain images to estimate regional CBF. The 3DSRT template uses the algorithm of SPM12 software to standardize anatomical coordinates based on those of the MNI. The ROI of 3DSRT was categorized into 12 segments: callosomarginal, precentral, central, parietal, angular, temporal, posterior cerebral, pericallosal, lenticular nucleus, thalamus, hippocampus, and cerebellum (Figure 1). 3DSRT's is allowed by Regulation for Enforcement of the Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices in Japan.

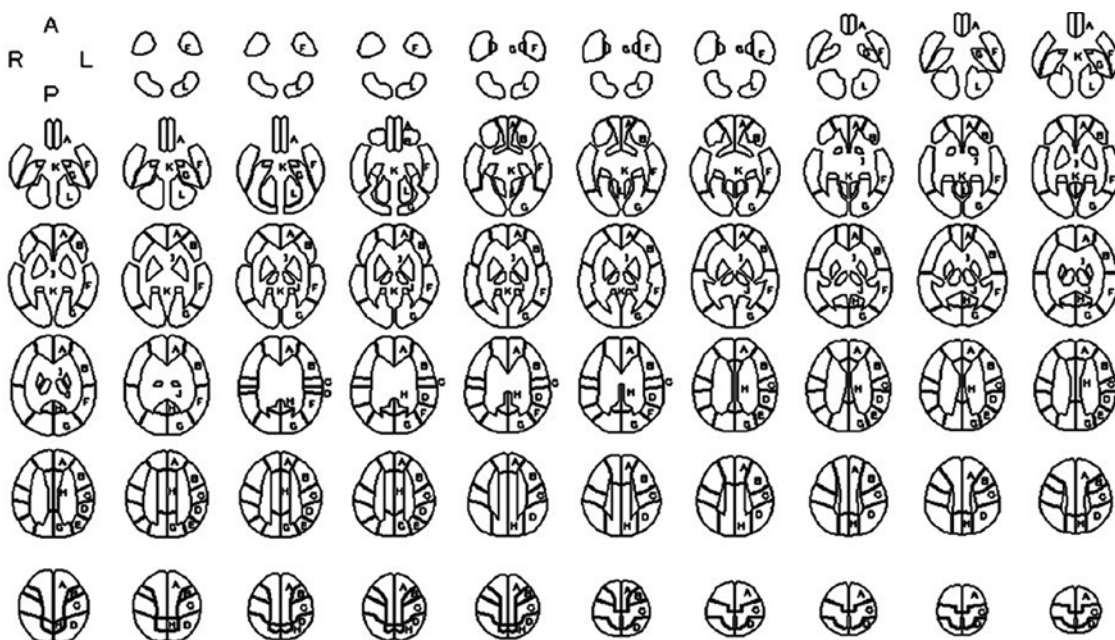


Figure 1

Statistical analysis

qCBF maps in the follicular and luteal phases of the female volunteers were also compared via a pixel-by-pixel comparison with SPM. $P < 0.05$ was considered significant. Reproducibility was evaluated by the intraclass correlation coefficient (ICC).

RESULTS

The mean qCBF values of all participants were 57.8 mL/100 g/min at the initial measurement and 55.1 mL/100 g/min at the second measurement, giving an ICC of qCBF in the 16 participants of 0.92. The mean qCBF values of the 8 men were 47.57 ± 2.70 mL/100 g/min at the first measurement and 48.46 ± 1.98 mL/100 g/min at the second measurement, which was not significantly different ($P = 0.36$). The mean qCBF values in the 8 women were 68.13 ± 2.95 mL/100 g/min in the follicular phase and 61.68 ± 5.06 mL/100 g/min in the luteal phase; the difference was statistically significant ($P < 0.05$; Table 1). The ICC of the corrected qCBF of all participants was 0.92, 0.94 in men, and 0.90 in women. The ICC of transit delay times was at 0.97, which was higher than that of qCBF, even in women (Table 2).

Statistical analysis of the regional differences by automatic ROI analysis revealed differences in qCBF between women and men in all of the cerebral regions, with the smallest difference found in the lentiform nucleus. In the follicular phase, the average qCBF values were higher in all of the cerebral regions, but especially in the callosomarginal and precentral (Figure 2). Transit flow time was shorter in women than in men in all cerebral regions (Figure 3).

Via a pixel-by-pixel comparison between men and women using SPM, differences in qCBF were found in the bilateral

inferior frontal gyrus, inferior temporal gyrus, and lower cerebellum ($P < 0.05$; Figure 4). In addition, significant differences in the transit flow time between men and women were shown in the right frontal lobe, parietal lobe, and inside of the cerebellum ($P < 0.05$; Figure 5). Finally, SPM analysis of the qCBF between the follicular and luteal phases revealed an increase in the bilateral frontal poles in the follicular phase versus the luteal phase ($P < 0.01$; Figure 6).

Table 1. Estimated average corrected CBF values and delay times.

| | Corrected CBF (mL/100 g/min) | Delay time (ms) |
|-------------------------------|------------------------------|-----------------|
| All participants, first scan | 57.85 ± 2.83 | 1377.62 ± 36.15 |
| All participants, second scan | 55.10 ± 3.52 | 1367.11 ± 40.28 |
| Men, first scan | 47.57 ± 2.70 | 1449.24 ± 44.93 |
| Men, second scan | 48.46 ± 1.98 | 1413.33 ± 45.15 |
| Follicular phase | 68.13 ± 2.95 | 1306.00 ± 27.36 |
| Luteal phase | 61.68 ± 5.06 | 1320.88 ± 35.41 |

Table 2. ICC repeatability of the quantitative values according to sex.

| ICC | Corrected CBF (95% CI) | Delay time (95% CI) |
|------------------|------------------------|---------------------|
| All participants | 0.92 (0.85-0.96) | 0.97 (0.93-0.98) |
| Men | 0.94 (0.88-0.98) | 0.94 (0.87-0.97) |
| Women | 0.90 (0.77-0.95) | 0.96 (0.91-0.98) |

ICC, intraclass correlation coefficient

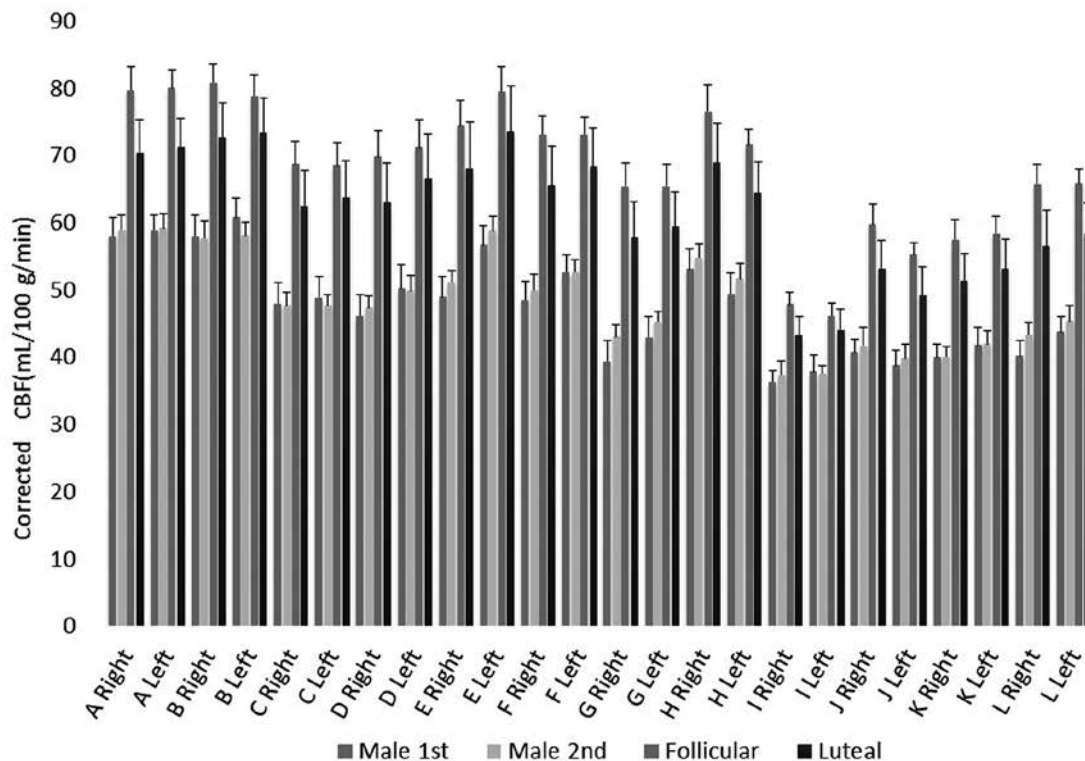


Figure 2

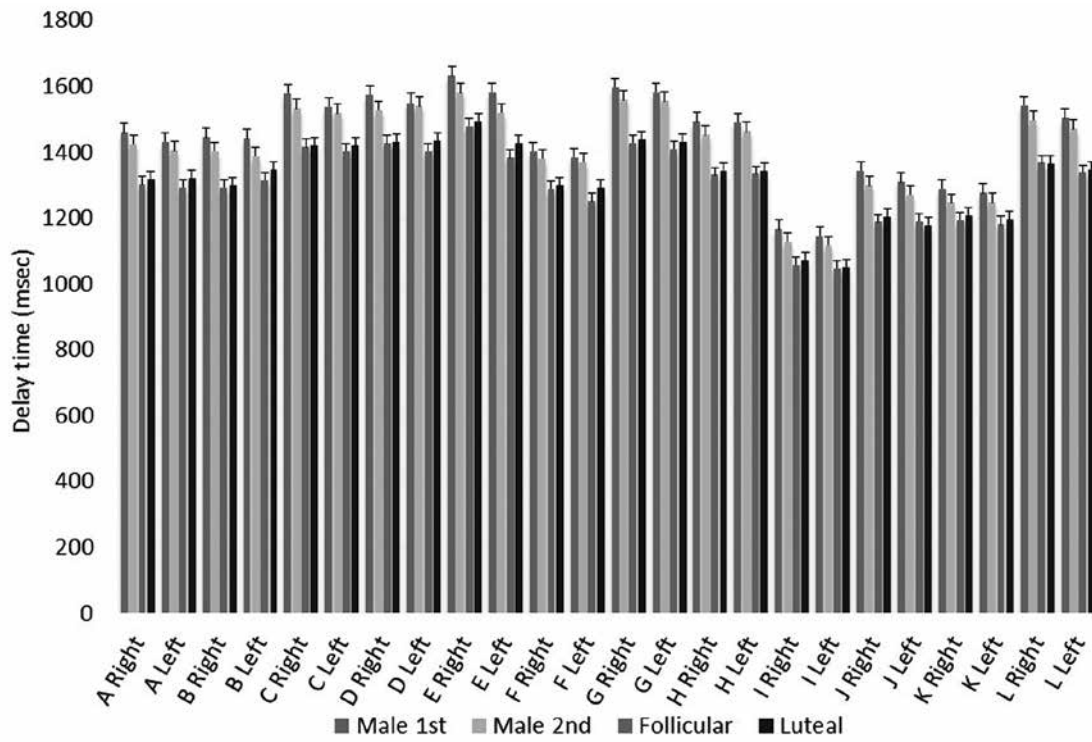


Figure 3

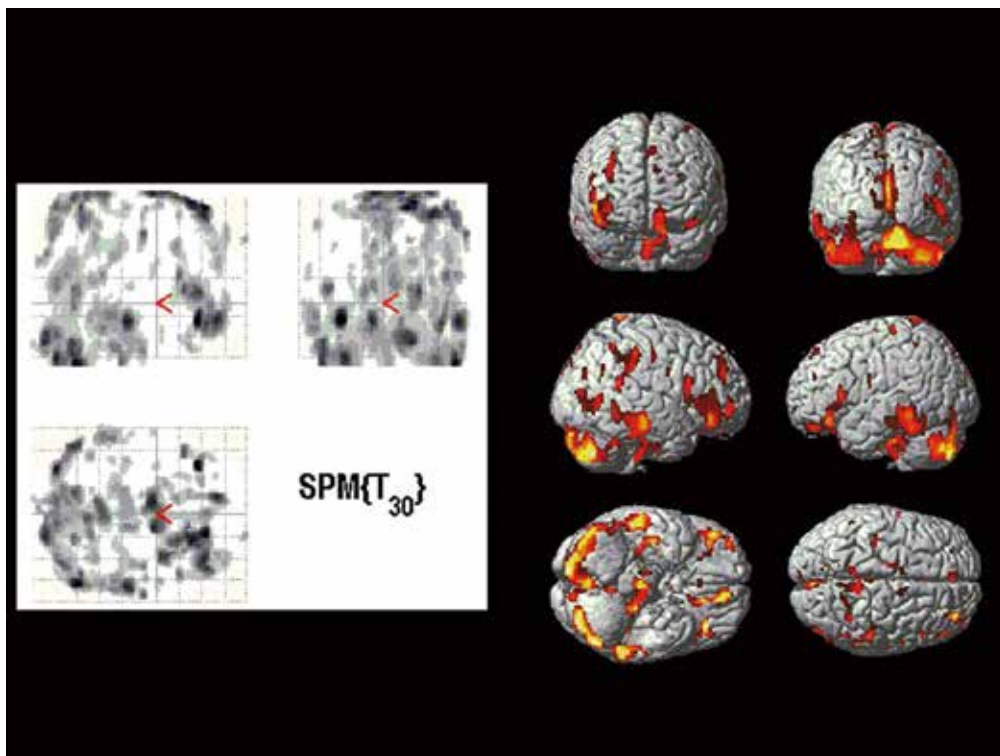


Figure 4

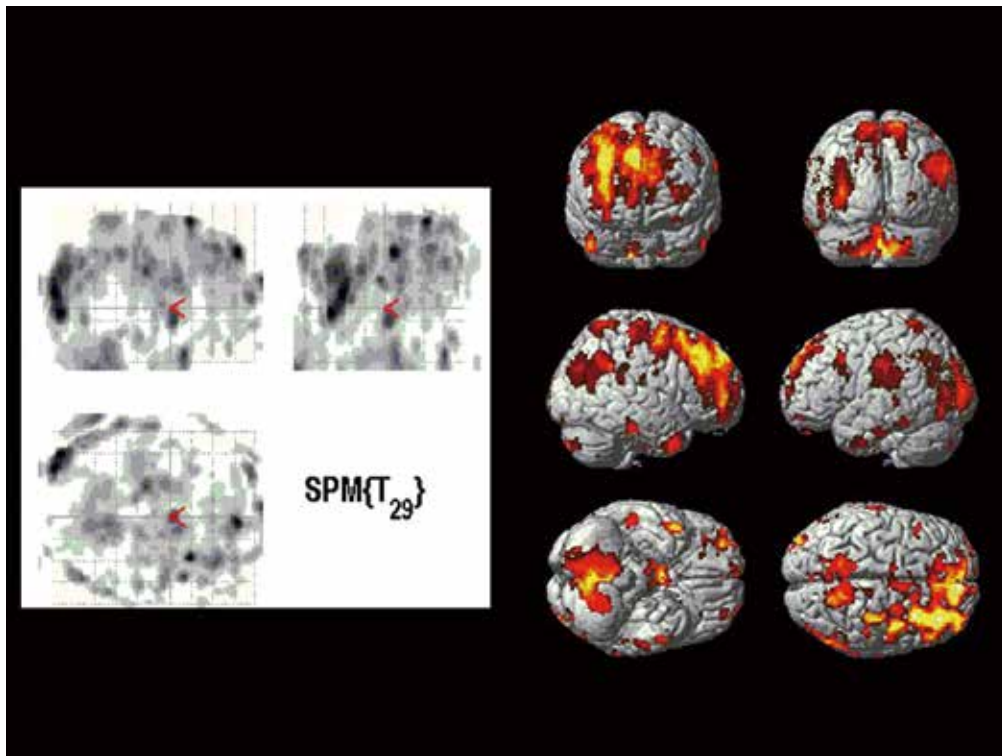


Figure 5

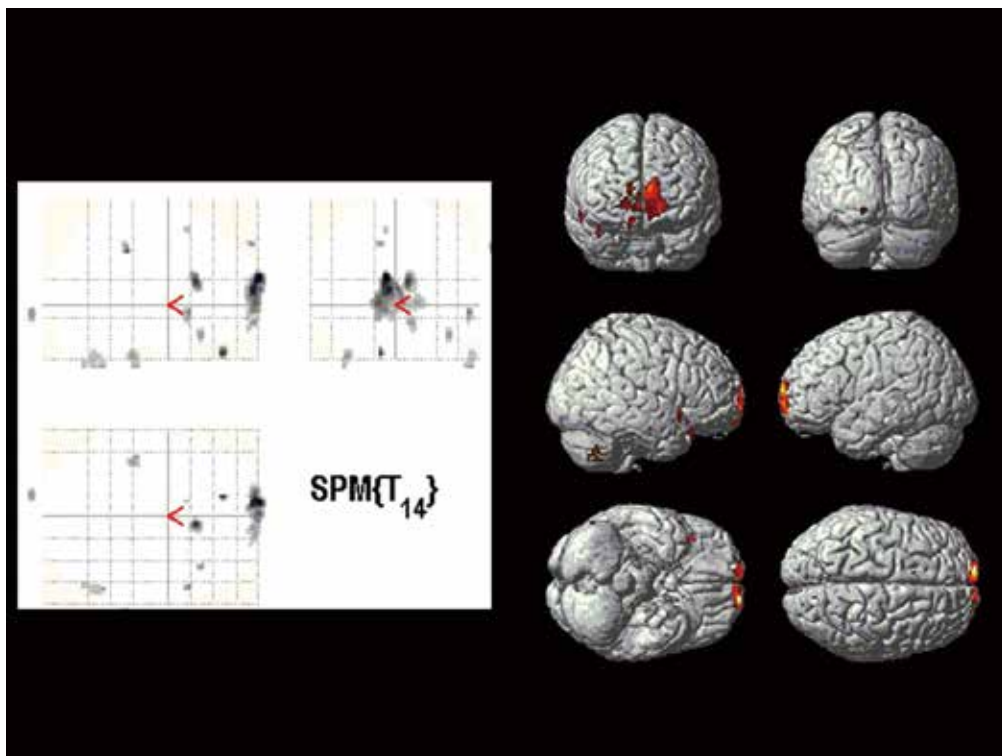


Figure 6

DISCUSSION

Kilroy *et al.* (7) examined the reliability of 2D and 3D pCASL with a single PLD time of 1.5 s in elderly populations, determining ICCs of 0.707 with 3D GRASE pCASL and of 0.362 with 2D EPI pCASL. Pfefferbaum *et al.* (16) reported high repeatability with an ICC of 0.961, globally normalized and evaluated by 3D pCASL with a PLD time of 2 s. Wu *et al.* (17) compared the reliability of 3D pCASL according to different PLD times and found that the ICCs of total ROIs were better with a PLD of 2.5 s than with a PLD of 1.5 s. In addition, they showed that the qCBF reliability depended on the regional ROI and produced ICCs of qCBF ranging from 0.44 to 0.95. In our study, the mean ICC of all participants with multi-delay 3D qCASL was 0.89, and the reproducibility was within that of the previous reports with a single PLD time, which was well maintained for the clinical evaluation, despite qCBF correction by transit flow time. This good reproducibility might be supported by the high ICC of transit flow time measured by three different PLD times. Furthermore, our study showed that the ICC of qCBF was lower in women than in men, indicating a larger variation in qCBF in women.

A comparison of qCBF values between men and women revealed that the qCBF value was higher in women than in men, which is consistent with the results of a dynamic PET study using [¹⁵O] H₂O emission recording by Aanerud *et al.* (9). Hermes *et al.* (10) also showed higher CBF in women (average age, 24.5 ± 2.3 years) than in men using a 1.5-T CASL MRI. From these results, it was generally considered that qCBF is higher in young adult women than in age-matched men. Furthermore, our study showed a difference in qCBF in women between the follicular and luteal phases. The low ICC and large variation in qCBF in women may be due to normal brain physiology.

Gur *et al.* (18) described higher CBF rates, a higher percentage of gray matter tissue, and higher interhemispheric connectivity in women than in men in their review and noted that these sex differences become more prominent with adolescence, perhaps due to puberty. It was speculated that the higher qCBF in young women might be due to a higher level of estrogens. Chang *et al.* (19) demonstrated that estradiol increased the activities of prostaglandin cyclooxygenase and prostaglandin synthetase, with the increased prostacyclin bioreactivity related to the qCBF increase in the follicular cycle in women. In our study, the cerebral regions with an increased qCBF in women were mainly in the bilateral inferior frontal lobe, including Broca's area. These areas may be associated with performance and word generation. In the follicular phase, the dominant area with a qCBF increase was the frontal pole, which may be related to prediction or planning, even though the function of the frontal pole is not well understood. Previous functional MRI studies have reported that brain activation in women is significantly associated with estradiol in the frontal and parietal regions. (20) Women with high estradiol levels have also been reported to alleviate psychosocial stress (21), and gender differences in the stress response circuit are regulated hormonally rather than genetically through the influence of subcortical brain activity on cortical control of alertness. (22,23) Changes in the localization of brain function due to increases and decreases in female hormones can be expected to affect certain local neurons and networks. The changes in blood flow in the brain due to the estrous cycle in our study may reflect the difference in the degree of influence of female hormones in the brain.

As far as we know, our study is the first to demonstrate differences in qCBF during the menstrual cycle.

On the other hand, we could not exclude the possibility that the sex difference might be partly due to differences in T1 and/or other hypothesized parameters in the multi-delay 3D pCASL

technique between men and women. For example, the labeling effect might differ depending on the thickness of the soft tissue in the neck between men and women, and cerebral blood flow in men might be underestimated due to the difference in cerebral volume. Another limitation was the lack of the quantitation of female hormones and hemoglobin because the appropriate consent to blood sampling could not be obtained from the participants. All female reproductive hormones exhibited significant circadian rhythms during the follicular phase. In contrast, only follicle stimulating hormone (FSH) and sex hormone binding globulin (SHBG) were significantly rhythmic during the luteal phase. The acrophase occurred in the morning for progesterone (P4); in the afternoon for FSH, luteinizing hormone (LH), and SHBG; and during the night for estradiol (E2). (24) Since we measured at almost a fixed time in the afternoon, we believe that the effect of the daytime rhythm is small.

The other limitation in our study was the small number of subjects, and further study including more subjects with various ages will be needed to confirm the consideration derived from our results.

CONCLUSION

This study showed good reproducibility of qCBF values measured by multi-delay pCASL and a sex difference in qCBF between young men and women. The qCBF in the inferior frontal gyrus was higher in women than in men and the qCBF in the frontal pole was higher in the follicular phase than in the luteal phase. Multi-delay pCASL is able to reveal physiological and sex differences in cerebral perfusion.

DISCLOSURE OF CONFLICTS OF INTEREST

Masafumi Harada received a research grant from Neurological and Psychiatric Disorders of the NCNP. Other authors declare that they have no conflicts of interest.

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