PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Hypometabolism in the supplementary and anterior cingulate cortices is related to dysphagia in Parkinson's disease: a cross-sectional and 3-year longitudinal cohort study
AUTHORS	Kikuchi, Akio; Baba, Toru; Hasegawa, Takafumi; Kobayashi, Michiko; Sugeno, Naoto; Konno, Masatoshi; Miura, Emiko; Hosokai, Yoshiyuki; Ishioka, Toshiyuki; Nishio, Yoshiyuki; Hirayama, Kazumi; Suzuki, Kyoko; Aoki, Masashi; Takahashi, Shoki; Fukuda, Hiroshi; Itoyama, Yasuto; Mori, Etsuro; Takeda, Atsushi

VERSION 1 - REVIEW

REVIEWER	Kenichi Kashihara MD, PhD Head, Department of Neurology, Okayama Kyokuto Hospital, Japan I have no conflicts of interests associated with the present study.
REVIEW RETURNED	11-Nov-2012

GENERAL COMMENTS	This study tried to fine the cortical areas responsible to dysphagia in patients with Parkinson's disease (PD) by use of FDG-PET. And found that hypometabolism of the SMA and ACC are associated with dysphagia. These results suggest both volitional and autonomic components of cortical function associated with swallowing are impaired in PD patients with dysphagia. Though the objective evaluation of swallowing function by videofluolometry may strengthen the significance of study, these results still are interesting enough to accept for publication. While reviewing, I got several questions of these other readers also may have. Major points The SMA may be attributed to the control of internally generated movements and complex sequences of movement. Was there any other motor symptom, such as gait freezing, impaired in parallel with SMA hypometabolism? Authors considered that the ACC may be attributed to the autonomic component of swallowing. Results of autonomic dysfunction such as constipation and orthostatic hypotension are correlated with ACC hypometabolism? Do the MSA and ACC hypometabgolisms correlated each other? Authors mentioned in introduction that the dysphagia in PD is thought to reflect impaired function of the medullary swallowing center. Functioning of this area is detectable by FDG-PET ? In the longitudinal study, was there any patient who developed dysphagia in three years? If any, add the results of FDG-PET study of them.

	Minor point Introduction: l 37-38, Provide a reference for ”dysphagia in PD is thought to reflect impaired function of the medullary swallowing center”.

REVIEWER	Suzuki, Masahiko Jikei University, Katsushika Medical Center, Neurology
REVIEW RETURNED	19-Nov-2012

THE STUDY	1. Regarding presence or absence of dysphagia The item on swallowing in Part II of the UPDRS allocates a score of zero for absence of dysphagia and a score of 1 (rare choking) or higher for presence of dysphagia. However, this is not an appropriate method of classifying subjects because the criteria are vague and lack objectivity. Many patients with Parkinson’s disease have silent aspiration (aspiration without choking), which is not reflected in these diagnostic items. I recommend classifying subjects based on the results of videofluorography regarding the presence or absence of aspiration or a history of pneumonia or asphyxia. 2. Assessment of swallowing The authors assessed swallowing based on the time to initiation of swallowing and the number of swallowing movements during a 30-second period. This assessment method evaluates the ease with which swallowing occurs, but not the “quality” of swallowing. With this assessment method, it is not possible to determine whether or not aspiration occurs or whether or not the saliva remains in the pharynx. The method of measuring the time to initiation of swallowing also is not clear. In order to accurately measure this, I believe methods such as surface electromyography should be used. 3. Assessment of swallowing and PET The authors noted that blood flow in the SMA and ACC decreased in patients in whom saliva swallowing did not readily occur. This is a plausible result, and appears to match the sites activated during saliva swallowing in healthy individuals as reported by Soros P. et al (Hum Brain Mapp 2009; 30: 2426-39). However, these activation sites are associated with saliva swallowing, and may not be as important for swallowing water. Swallowing is performed by reflex as long as the brain stem is intact. Saliva swallowing lacks sensory input from the pharynx, and occurs due to input from the cerebrum, a process that involves the SMA and SCC. What is in question here is the connection between individuals performing poorly on the saliva swallowing task and clinical dysphagia. The present study may cover only one part of swallowing.
GENERAL COMMENTS	The authors have a good objective, and their study is original. The results are plausible, but it would be a stretch to explain dysphagia associated with Parkinson’s disease based on this study.

REVIEWER	Dr Emilia Michou, PhD, PGDip FMHS Stepping Stones Fellow University of Manchester Gastrointestinal Centre

	Institute of Inflammation and Repair Faculty of Medical and Human Sciences University of Manchester Clinical Sciences Building Salford Royal Hospital (part of the Manchester Academic Health Sciences Centre (MAHSC)) Eccles Old Road
REVIEW RETURNED	20-Nov-2012

THE STUDY	The title of the paper is too ambitious. However, the study is important for the delineation of the underlying mechanisms of dysphagia in PD. There are several sentences that need rephrasing (i.e. page 5, line 20). In addition, information should have been added in the paper that the authors have compared glucose metabolism and whether they obtained whole-brain or cortical data only. PET scanning method should have been explained. There is no reference as to whether PET was during rest or active swallowing. In particular, there is information missing about: a) withdrawal from stimulants 12 hours prior to scanning, b) how long after the administration of FDG was the scan performed,c) if scans were performed during active swallowing, how were the scans matched across subjects,d) time of the scanning time with respect to PD medication (wearing-off symptoms?), e) field of view and whether there were 2D or 3D emmision scans. Evaluation of swallowing: a) when was it performed, b) there is no explanation for the manner that swallowing initiation delay was measured (i.e. did you give a bolus? Used a timer? What was the starting point to measure delay – tongue movement? I cannot find the reference for standardization of this procedure and therefore the conclusion to use this in the clinical setting is vague assumption. Also, it is of interest (and not discussed) that the 30 seconds swallowing assessment was not affected by the delay in swallowing initiation in PD patients. It would have been interesting to have behavioral measurements from healthy volunteers with your outcome measures for swallowing behavior. The authors did not check dysphagia, but swallowing behavior, since no Videofluoroscopy was performed. The measurements for swallowing behavior they used somewhat 'contaminated' with the oral and buccal motor bradykinesia that the patients may present (not in the discussion). In the absence of any diagnostic and assessment marker and/or inclusion criteria for dysphagia in the PD patients studied, the results and discussion are lacking power.
RESULTS & CONCLUSIONS	Results should have been viewed according to previous PET studies in swallowing. PET scans of the healthy individuals should have been added. An important methodological issue here is that the hyper-metabolism (obvious in authors' images) are not discussed and analytically explained in the results section. One valuable question is that is whether the hyper-metabolism should be reviewed together with the hypometabolism, since this would have provided information about the compensatory mechanisms in PD. Also, there was no comparison of the change in the PD patients profile within groups for the LDopa dose (there was a considerable increase) and there is no discussion about the change in UPDRS scores in PD patients at 3 years follow-up within groups. Additional medication intake (i.e apomorphine) is missing. Also missing from the discussion the changes in brainstem areas. There are several overstatements, i.e. the use of the time test for dysphagia changes, the use of tongue training in dysphagic population. It is clearly an overstatement to consider relationship to dysphagia, when there is

	no cut-off point or standardization for the outcome measures the authors used. Discussions should have included several other parameters that changed within the 3 year follow-up period in PD patients.

VERSION 1 – AUTHOR RESPONSE

Responses to Dr. Kenichi Kashihara

1. The SMA may be attributed to the control of internally generated movements and complex sequences of movement. Was there any other motor symptom, such as gait freezing, impaired in parallel with SMA hypometabolism?

In the present study, no significant correlations were found between other motor symptoms such as gait freezing and the degree of hypometabolism in SMA (uncorrected $p<0.001$).

2. Authors considered that the ACC may be attributed to the autonomic component of swallowing. Results of autonomic dysfunction such as constipation and orthostatic hypotension are correlated with ACC hypometabolism?

In the group comparison between PD with dysphagia and that without dysphagia, no differences were found in autonomic dysfunction such as constipation or orthostatic hypotension.

3. Do the SMA and ACC hypometabgolisms correlated each other?

Their hypometabolisms were correlated with each other.

4. Authors mentioned in introduction that the dysphagia in PD is thought to reflect impaired function of the medullary swallowing center. Functioning of this area is detectable by FDG-PET?

We could not detect medullary hypometabolism at baseline nor after a 3-year follow-up period (uncorrected $p<0.001$, figures 3, 4). We added a sentence in the results section (page 9, lines 7-8).

5. In the longitudinal study, was there any patient who developed dysphagia in three years? If any, add the results of FDG-PET study of them.

During the 3-year follow-up period, four patients complaind of dysphagia. These patients showed a tendency for regional hypometabolism in SMA and ACC compared to normal controls (uncorrected $p<0.001$). However, the number of patients seemed to be insufficient in the analysis for group comparison.

6. Introduction: l 37-38, Provide a reference for “dysphagia in PD is thought to reflect impaired function of the medullary swallowing center”.

We added a reference (Hunter PC, et al. J Neurol Neurosurg Psychiatry 1997; 63:579-583) (page 4, line 15).

Responses to Dr. Masahiko Suzuki

1. Regarding presence or absence of dysphagia The item on swallowing in Part II of the UPDRS allocates a score of zero for absence of dysphagia and a score of 1 (rare choking) or higher for

presence of dysphagia. However, this is not an appropriate method of classifying subjects because the criteria are vague and lack objectivity. Many patients with Parkinson's disease have silent aspiration (aspiration without choking), which is not reflected in these diagnostic items. I recommend classifying subjects based on the results of videofluorography regarding the presence or absence of aspiration or a history of pneumonia or asphyxia.

We agree with the reviewer's opinion. Parkinson's disease without choking may have silent aspiration and videofluorography is important in evaluating whether or not silent aspiration occurs (Bushmann M, et al. Neurology 1989; 39:1309-1314). However, even if patients without choking have silent aspiration, the degree may be mild and such silent aspiration is rarely associated with serious respiratory infection (Wintzen AR, et al. Can J Neurol Sci 1994; 21:53-56). In fact, in the present study, percutaneous endoscopic gastrostomies or feeding tube were needed in 3 of 9 patients with choking because of aspiration pneumonia within 4 years from the baseline, although only in 1 of 18 patients without choking required tube feeding. We think that the complaint of choking in Parkinson's disease is related to aspiration that is of considerable clinical importance.

2. Assessment of swallowing
The authors assessed swallowing based on the time to initiation of swallowing and the number of swallowing movements during a 30-second period. This assessment method evaluates the ease with which swallowing occurs, but not the "quality" of swallowing. With this assessment method, it is not possible to determine whether or not aspiration occurs or whether or not the saliva remains in the pharynx. The method of measuring the time to initiation of swallowing also is not clear. In order to accurately measure this, I believe methods such as surface electromyography should be used.

Surface electromyography might be a better method of measuring the time to initiate swallowing. However, the time needed for swallowing initiation and swallowing frequency for 30 seconds by our methods showed high reproducibility. Therefore, we believe that our methods were objective. We added a sentence in the discussion section (page 11, lines 10-11).

3. Assessment of swallowing and PET
The authors noted that blood flow in the SMA and ACC decreased in patients in whom saliva swallowing did not readily occur. This is a plausible result, and appears to match the sites activated during saliva swallowing in healthy individuals as reported by Soros P. et al (Hum Brain Mapp 2009; 30: 2426-39). However, these activation sites are associated with saliva swallowing, and may not be as important for swallowing water. Swallowing is performed by reflex as long as the brain stem is intact. Saliva swallowing lacks sensory input from the pharynx, and occurs due to input from the cerebrum, a process that involves the SMA and ACC. What is in question here is the connection between individuals performing poorly on the saliva swallowing task and clinical dysphagia. The present study may cover only one part of swallowing.

Sörös P, et al. (Hum Brain Mapp 2009; 30: 2426-2439) reported that, using an activation likelihood estimation meta-analysis, clusters with higher activation likelihood for saliva than for water swallowing were found in the bilateral SMA and ACC. This study, however, is a meta-analysis and each report was evaluated by different methods such as PET (FDG or H2O), functional MRI (4.0T or 1.5T) and MEG. On the other hand, Martin R, et al. (Exp Brain Res 2007; 176: 12-22) reported that, using the same condition of functional MRI in saliva and water swallowing, water swallowing activated more expansive SMA and ACC areas compared with saliva swallowing. We do not think that SMA and ACC functions are related to saliva swallowing alone. In the present study, the time needed for swallowing initiation was above average in 4 of 8 patients with dysphagia and percutaneous endoscopic gastrostomies were performed in 2 of these 4 patients within 4 years of the baseline study. By measuring the time needed for swallowing initiation, we may be able to predict the long-term prognosis of dysphagia. Therefore, we think that the swallowing initiation delay is related to general

swallowing function.

Responses to Dr Emilia Michou

1. There are several sentences that need rephrasing (i.e. page 5, line 20).

We rephrased dysphasia as swallowing difficulty (page 4, line 7).

2. In addition, information should have been added in the paper that the authors have compared glucose metabolism and whether they obtained whole-brain or cortical data only.

Global normalization was performed using SPM's ''proportional scaling,'' and proportional threshold masking was set at 0.8. We added this sentence in the data analysis section (page 6, lines 10-11).

3. PET scanning method should have been explained. There is no reference as to whether PET was during rest or active swallowing.

The PET scanning was performed under resting conditions. We added a phrase in the PET procedure section (page 5, lines 18-19).

4. In particular, there is information missing about: a) withdrawal from stimulants 12 hours prior to scanning, b) how long after the administration of FDG was the scan performed, c) if scans were performed during active swallowing, how were the scans matched across subjects, d) time of the scanning time with respect to PD medication (wearing-off symptoms?), e) field of view and whether there were 2D or 3D emmision scans.

a) We administered anti-parkinsonian drugs to the PD patients, but not other potential stimulants. b) After a FDG-uptake period of 1 hour, scans of 10 minutes were acquired. c) The scans were performed at rest. d) We performed the scans during the "on" state without L-dopa induced dyskinesia. e) The field of view was 340mm and scanning was performed in the 3D mode. We added some phrases in the PET procedure section (page 5, lines 17-20 and page 6, line 2).

5. Evaluation of swallowing: a) when was it performed, b) there is no explanation for the manner that swallowing initiation delay was measured (i.e. did you give a bolus? Used a timer?

a) We evaluated swallowing function during the "on" state without L-dopa induced dyskinesia. b) We used a timer. We added some phrases (page 5, lines 6-7 and page 6, line 23).

6. What was the starting point to measure delay – tongue movement?

The verbal signal to start swallowing was the starting point. We added a phrase in the evaluation of swallowing section (page 6, line 22).

7. I cannot find the reference for standardization of this procedure and therefore the conclusion to use this in the clinical setting is vague assumption.

Surface electromyography might be a better method of measuring the time to initiate swallowing. However, the time needed for swallowing initiation and swallowing frequency for 30 seconds showed high reproducibility. We think that our method is a new approach to assessing the swallowing function. We added a sentence in the discussion section (page 11, lines 10-11).

8. Also, it is of interest (and not discussed) that the 30 seconds swallowing assessment was not affected by the delay in swallowing initiation in PD patients. It would have been interesting to have behavioral measurements from healthy volunteers with your outcome measures for swallowing behavior.

We measured the 30 seconds swallowing frequency in 10 healthy volunteers and the mean swallowing frequency for 30 seconds was 5.10±2.42. We added a phrase in the evaluation of swallowing section (page 6, line 19) and a sentence in the results section (page 8, lines 1-3).

9. The measurements for swallowing behavior they used somewhat 'contaminated' with the oral and buccal motor bradykinesia that the patients may present (not in the discussion).

No significant difference was found in the UPDRS motor score between PD with and without dysphagia after a 3-year follow-up. In spite of the result, the time needed for swallowing initiation was worsening in PD with dysphagia. We think that bradykinesia is not directly related to the outcome measurement results for evaluation of swallowing. We added some sentences in the discussion section (page 9, line 24 – page 10, line 4).

10. In the absence of any diagnostic and assessment marker and/or inclusion criteria for dysphagia in the PD patients studied, the results and discussion are lacking power.

The diagnostic and assessment marker for dysphagia might be insufficient in this study. However, PD patients with swallowing initiation delay at baseline showed higher frequency of percutaneous endoscopic gastrostomies within 4 years. Therefore, we believe that the swallowing initiation delay is related to dysphagia in PD.

11. Results should have been viewed according to previous PET studies in swallowing.

We reviewed according to previous PET studies on swallowing (page 9, line 14).

12. PET scans of the healthy individuals should have been added.

The FDG-PET scans were performed in 10 age-matched control subjects and compared with PD with dysphagia or without dysphagia. We added a sentence in the PET procedure section (page 5, lines 15-16).

13. An important methodological issue here is that the hyper-metabolism (obvious in authors' images) are not discussed and analytically explained in the results section.

No regional hypermetabolism was found in the PD patients with dysphagia or without dysphasia compared with the normal control subjects at baseline. After the 3-year follow-up period, only a small degree of hypermetabolism in the left middle and right superior occipital lobes, left middle temporal lobe, left supramarginal gyrus, and left calcarine cortex in PD patients with dysphagia and in the left supramarginal gyrus, left postcentral gyrus, and left middle and superior lobes in PD patients without dysphagia was found compared with the normal control subjects. We added some sentences in the results section (page 8, lines 20-23 and page 9, lines 3-7).

14. One valuable question is that is whether the hyper-metabolism should be reviewed together with the hypometabolism, since this would have provided information about the compensatory mechanisms in PD.

The comment is very important. We think that there were not sufficient compensatory mechanisms for

swallowing difficulty in PD because regional hypermetabolism was not found in PD with dysphagia and without dysphagia at baseline. We added some sentences in the results and discussion sections (page 8, lines 20-23 and page 9, lines 3-7) (page 10, lines 4-6).

15. Also, there was no comparison of the change in the PD patients profile within groups for the LDopa dose (there was a considerable increase) and there is no discussion about the change in UPDRS scores in PD patients at 3 years follow-up within groups.

There was no significant difference in the L-dopa equivalent dose between baseline and 3-year follow-up in PD with dysphagia using paired t-test ($p>0.05$), while a significant difference was found in PD without dysphagia ($p<0.05$). No significant differences were found in the UPDRS motor score between baseline and 3-year follow-up within groups using paired t-tests ($p>0.05$). We added some phrases and sentences in the results and discussion sections (page 8, lines 3-7) (page 9, lines 19-20 and page 9, line 24 - page 10, line 4).

16. Additional medication intake (i.e apomorphine) is missing.

We did not use other medications including apomorphine.

17. Also missing from the discussion the changes in brainstem areas.

We could not find any changes in the brainstem areas.

18. There are several overstatements, i.e. the use of the time test for dysphagia changes, the use of tongue training in dysphagic population.

We agree that the use of the time test for dysphagia changes and tongue training in dysphagic population may be overstatements. We modified the phrases (page 11, line 19 and page 11, line 24 - page 12, line 2).

19. It is clearly an overstatement to consider relationship to dysphagia, when there is no cut-off point or standardization for the outcome measures the authors used.

We measured the time needed for swallowing initiation and the 30 seconds swallowing frequency in 10 healthy volunteers. The results were almost the same as those of PD without dysphagia. We added these results (page 8, lines 1-3).

20. Discussions should have included several other parameters that changed within the 3 year follow-up period in PD patients.

We added a discussion on several other parameters such as UPDRS motor score and L-dopa equivalent dose that changed within the 3-year follow-up period in PD patients (page 9, line 24 - page 10, line 6).

VERSION 2 – REVIEW

REVIEWER	Kenichi Kashihara Head, Department of neurology, Okayama Kyokuto Hospital, Japan I declare no competing interests with respect to the present article.
REVIEW RETURNED	31-Dec-2012

THE STUDY	P5 l6: Sentence describing "evaluation of swallowing, and PET studies were evaluated" seems strange. Maybe because authors used terms of the same origin for both the subject (evaluation) and predicate (evaluated). "Evaluation of swallowing" can be substituted by "swallowing", "swallowing function", "swallowing difficulty", etc.
GENERAL COMMENTS	This manuscript was revised almost adequately and is acceptable.