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Original Article

First Clinical Application of Octacalcium Phosphate Collagen Composite in Human Bone Defect

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We have previously demonstrated that octacalcium phosphate (OCP) collagen composite (OCP/collagen) promotes bone regeneration in a critical-sized bone defect of a rodent or canine model. This study was designed to investigate the bone regeneration of OCP/collagen in human bone defect as a first clinical trial. Two patients who had a radicular cyst or apical periodontitis consented to participate in our clinical study, and OCP/collagen was implanted into the defects after operation. Radiographic examination showed effective bone healing in each bone defect at 3 or 6 months. Likewise, computed tomography value significantly increased after implantation. Postoperative wound healing was uneventful, and neither infection nor allergic reaction against OCP/collagen was observed for the entire period. This study demonstrated that OCP/collagen would be safely used and enhanced bone regeneration in human bone defects. To reinforce the efficacy of OCP/collagen as a bone substitute material, it should be compared with other suitable comparators in the future.

Introduction

Reconstruction of large bone defects caused by extirpation of tumor and cyst is an important issue in the field of orthopedic and oral surgery.[1] Autologous bone includes osteoprogenitor cells, collagenous bone matrix, bone mineral, and growth factors, such as bone morphogenetic proteins.[2] This composition in autologous bone should promote the induction of new bone within bone defects if it is transplanted. However, there are problems associated with the use of autologous bone in terms of donor site pain, morbidity, infection, extra blood loss, and higher cost due to longer operation.[3]

Synthetic calcium phosphate has been widely used as a bone substitute of autologous bone, such as sintered ceramics of hydroxyaptite (HA), β-tricalcium phosphate (β-TCP), and calcium phosphate-based cements to form HA because it has no limitation in terms of availability, excellent osteoconductivity, and relatively uniform quality regardless of the production lot provided by manufacturers.[4–6] However, it is widely accepted that its bone regenerative properties are still inferior to those of autologous bone.[7]

Octacalcium phosphate (OCP) has been suggested to be a precursor of biological apatite, such as dentin, enamel, and bone. Indeed, the presence of OCP has been demonstrated in these types of biological apatite.[8–10] Our previous study indicated the superior osteoconductivity of synthetic OCP compared with that of amorphous calcium phosphate, dicalcium phosphate, Ca-deficient HA, and HA if implanted into the subperiosteal area of the calvaria of mice.[11] In addition, it has been confirmed that bone regeneration of synthetic OCP is higher than that of HA or β-TCP ceramic *in vivo*,[12] that synthetic OCP promotes osteoblastic cell differentiation while OCP is converted into HA *in vitro*,[13] and that the osteogenic effect is dependent on the amount of OCP.[14]

Although OCP possesses many desirable properties as a bone substitute, it cannot be molded using sintering processes because of its intrinsic crystal structure. To resolve the disadvantages, including the improvement of handling, OCP combined with atelocollagen (OCP/collagen) was prepared.[15] OCP/collagen significantly enhances bone regeneration more than OCP alone, collagen alone, and HA or β-TCP ceramic combined with collagen in rat calvarial bone defect,[15,16] and bone formation by OCP/collagen increases with the amount of OCP in the OCP/collagen.[17] Aiming at clinical application, bone regeneration by OCP/collagen was examined in canine models, and implantation of OCP/collagen significantly enhanced bone regeneration more than those of β-TCP, collagen, and untreated control at a critical-sized calvarial bone defect, a tooth extraction socket, or an alveolar cleft model.[18–21]

To the best of our knowledge, the present report is the first on the clinical evaluation of OCP/collagen. This new bone regenerative material was used for filling bone defects after cystectomy. The clinical evaluation of the two patients was conducted according to Japanese ethical guidelines and regulations. The first objective of this study was to investigate the safety of OCP/collagen for clinical use. The second objective was to investigate the efficacy of this material if implanted into a cyst cavity. Radiographic examination of the implanted sites was performed, and the findings were compared to those of the surrounding host bone up to 6 months after implantation.

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Materials and Protocols of the Clinical Trial

Preparation of OCP/collagen

OCP was prepared by mixing calcium acetate hydrate solution and sodium phosphate monobasic solution as described previously,[11] and using sterile-filtered water during the whole preparation. The precipitates were washed several times with sterile-filtered water and then lyophilized. Sieved OCP granules (particle sizes 300–500 μm) obtained from the dried OCP were sterilized by heating at 120°C for 2 h in dry condition. Our previous study showed that such heating does not affect physical properties, such as the crystalline structure or specific surface area of OCP granules, although it was reported that increasing the temperature above 100°C induced collapse of the OCP structure because of dehydration.[22] Powder X-ray diffraction (XRD) patterns of the obtained OCP were recorded using step-scanning at 0.05-degree intervals from 3.00 to 60.00 degrees, with Cu Kα X-rays on a diffractometer (Mini Flex; Rigaku Electrical Co., Tokyo, Japan) at 30 kV and 15 mA. The range of 2θ included the primary peak (100) reflection of OCP around 4.7 degrees. Collagen was prepared from NMP collagen PS (Nippon Meat Packers, Tsukuba, Ibaraki, Japan), and a lyophilized powder of pepsin-digested atelocollagen was isolated from porcine dermis. NMP collagen PS was dissolved in sterile-filtered water and adjusted to a final concentration of 3% at pH 7.4. OCP/collagen was prepared from NMP collagen PS and OCP granules. OCP was added to the concentrated collagen and mixed.[15] The weight percentage of OCP in OCP/collagen was 77%. This OCP/collagen mixture was then lyophilized and disks were molded (9 mm diameter, 1 mm thick) (Fig. 1). The molded OCP/collagen underwent dehydrothermal treatment (150°C, 24 h) in a Vacuum Drying Oven, DP32 (Yamato Scientific, Tokyo, Japan). The clinical batches were prepared aseptically. Two pieces of the molded OCP/collagen were placed in a sterilized microcentrifuge tube (509-GRD-SC; Quality Scientific Plastics, San Diego, CA), and the OCP/collagen-containing tube was then packed with Fisherbrand® instant sealing sterilization pouch (9×13 cm; Fisher Scientific, Pittsburgh, PA). The packed OCP/collagen was subsequently sterilized using electron beam irradiation (5 kGy) to make it ready-to-use. After sterilization, the XRD pattern derived from OCP/collagen indicated a collapsed and reduced primary (100) peak with a shift from 4.7 to 5.3 degrees at 2θ, as previously reported.[15]

Design of the clinical trials

This study is a part of the clinical study of "Bone regenerative therapy by OCP collagen composites," which was registered as JPRN-UMIN000004655 in the University Hospital Medical Information Network in Japan (UMIN) and International Clinical Trials Registry Platform Search Portal of the World Health Organization. The protocol of the clinical trial was submitted and approved by the research ethics committee of the Tohoku University Graduate School of Dentistry under reference number 20–27. The principal investigator and promoter was Prof. Shinji Kamakura (DDS, PhD), and this trial was performed at the Department of Oral and Maxillofacial Surgery, Tohoku University Hospital, Sendai, Japan.

This clinical trial is a single-arm nonrandomized intervention study, and 10 cases of tooth extraction socket and cyst cavity were studied. All patients signed a formal consent form and were finally included in the study. The aim of the first clinical evaluation in this study was to demonstrate the safety, by clinical examination and recording of adverse events, of using OCP/collagen to fill these defects. The second clinical evaluation focused on bone regeneration in these defects by radiographic examination. This study reported two cases of cyst cavity after implantation of OCP/collagen.

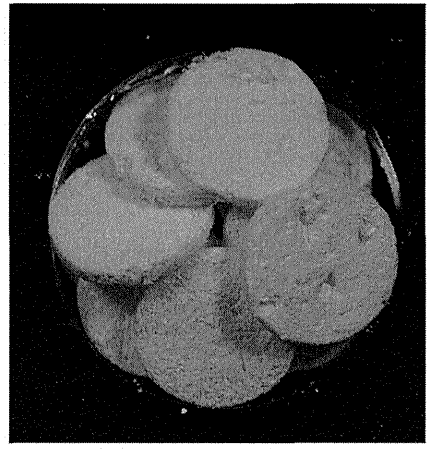

FIG. 1. A picture of OCP/collagen disks. OCP/collagen disk is 9 mm in diameter and 1 mm thick. OCP, octacalcium phosphate.

Clinical, laboratory, and radiographic examination

Dental radiography and clinical examination were performed before cystectomy, and 1 and 7 days, and 1, 3, and 6 months after OCP/collagen implantation. A computed tomography (CT) was made before the cystectomy and 3 or 6 months after implantation. Then, the value of CT was measured at the center of the bone defect. The clinical examination involved the observation of general and local conditions, including inflammatory symptoms in the operative site. In addition, laboratory examination was carried out before cystectomy, and 1 day, and 1, 3, and 6 months

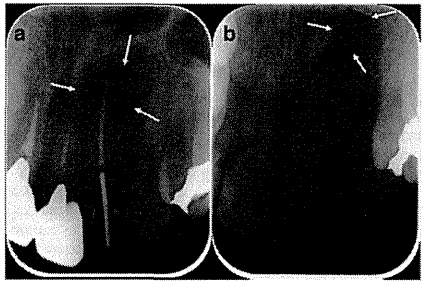

FIG. 2. X-ray pictures of patients. **(a)** Patient A: A radicular cyst at the apex of the left maxillary lateral incisor. **(b)** Patient B: A radicular cyst at the apex of the left maxillary central incisor. Arrows show the margin of the bone defect.

after implantation. This comprised factors such as peripheral blood figures, liver and kidney function, and urinalysis.

Case Reports

The first case (patient A) was a 37-year-old man and the second one (patient B) was a 23-year-old woman. Both patients consulted our hospital for the examination of radiolucent areas of anterior maxillary regions. Their medical histories contained nothing of relevance, and both patients were clinically diagnosed with radicular cyst of anterior maxilla by radiographic examination (Fig. 2a, b). The radiolucent figure of patient A was about 8 mm in diameter at the apical region of the left maxillary lateral incisor and that of patient B was about 5×5×10 mm at the apical region of the left maxillary central incisor. Under local anesthesia, 2% lidocaine hydrochloride with 1:80,000 epinephrine was injected into anterior maxillary region, and both patients underwent cystectomy (Fig. 3a, b). After irrigation, OCP/collagen disks were implanted in the defect. Four or six disks of OCP/collagen were used in the defect of each patient (Fig. 3c, d). Then, ablated gingiva and periosteum were repositioned and sutured with absorbable suture (4-0 Coated Vicryl™; Ethicon, Inc., Irvine, CA). Both cases were diagnosed as radicular cyst by postoperative pathological examination.

In both cases, postoperative wound healing was satisfactory, and there was no postoperative infection or allergic reaction at the operative site during the observation period. The height or width of alveolar bone was maintained until 6 months after OCP/collagen implantation (Fig. 4). Laboratory examination revealed no abnormal findings except that

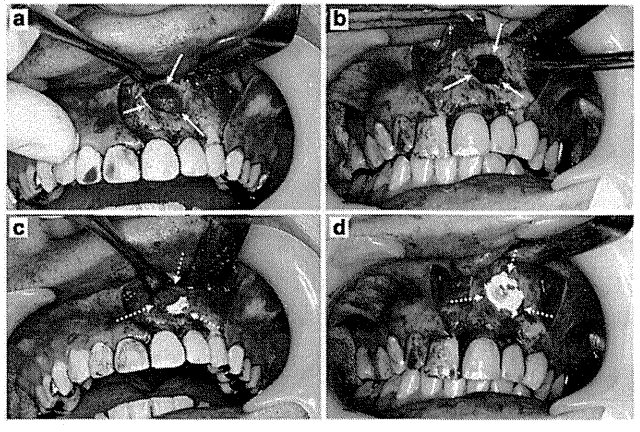

FIG. 3. Pictures of cystectomy and OCP/collagen disk implantation. Cystectomy of **(a)** patient A and **(b)** patient B. Arrows show the margins of these defects. Implantation of OCP/collagen disks at the defects of **(c)** patient A and **(d)** patient B. Dot arrows show OCP/collagen disks in these defects. Color images available online at www.liebertpub.com/tea

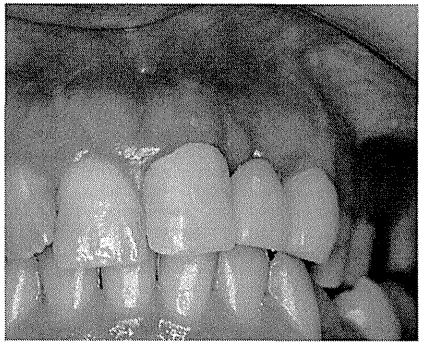

FIG. 4. Pictures of operative region in patient B at 6 months after OCP/collagen implantation. There was temporary crown restoration. Color images available online at www.liebertpub.com/tea

a slight increase of C-reactive protein was observed. In radiographic examination after the implantation of OCP/collagen, there was little radiopacity in the defect 1 day after the implantation (Fig. 5a, b). The radiopacity in the defect increased slightly at 3 months (Fig. 5c, d) and was further improved 6 months after the operation (Fig. 5e, f). Examination by CT scan showed that the defect of patient A or B was dotted with hard tissue-like bone at 3 months after implantation (Fig. 6c, d). At 6 months after OCP/collagen implantation, the density of the hard tissue was increased and the hard tissue spread in the defect (Fig. 6e, f). This hard tissue arose from the center of the defect as well as its margin.

The CT values of the radicular cysts before implantation were 21 Hounsfield units (HU) in patient A and 23 HU in patient B. The CT values of patient A were 327 and 527 HU at 3 and 6 months after OCP/collagen implantation, respectively. Similarly, those of patient B were 316 and 602 HU. The CT values increased significantly at 3 or 6 months compared with those before implantation (Table 1).

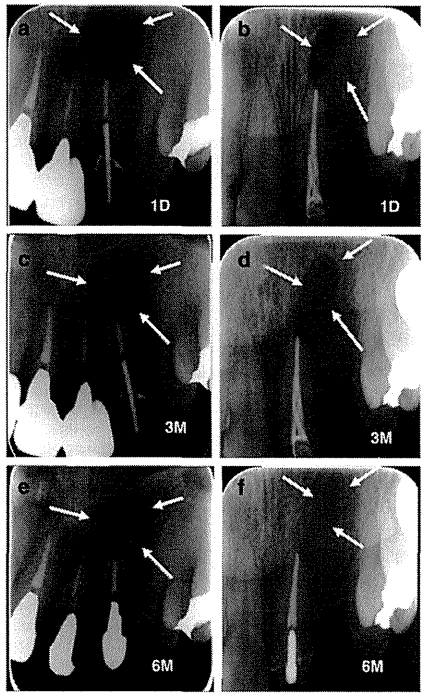

FIG. 5. X-ray pictures. **(a)** At 1 day after cystectomy in patient A and **(b)** patient B. **(c)** At 3 months after cystectomy in patient A and **(d)** patient B. **(e)** At 6 months after cystectomy in patient A and **(f)** patient B. Arrows show the margins of the bone defects.

Discussion

When OCP/collagen was implanted into bone defects after cystectomy, no postoperative infection or allergic symptom was observed for the entire period. In addition, no serious side effects were observed in this investigation. Laboratory examination showed no serious abnormal results in each patient, although C-reactive protein increased 1 day after OCP/collagen implantation as well as usual radicular cystectomy and recovered to its normal level at 7 days (data not shown). Although these patients complained of pain in the operative site for a few days, this symptom was controlled with a painkiller. In addition, postoperative wound swelling seemed to be limited compared with the operative stress. At 3 months after OCP/collagen implantation, the implanted site became hard on palpation. These results suggest that OCP/collagen would be safe to use if implanted into adult patients after cystectomy.

In radiographic examination, the radiopacity in the bone defect after OCP/collagen implantation increased with time. In addition, CT examination in the defect indicated dotted radiopacity at 3 months, which had increased and spread in the defect at 6 months. If OCP/collagen were implanted into a bone defect, the implanted OCP/collagen indicated radiopacity at several weeks after implantation, whereas OCP/collagen itself had little radiopacity.[14,15] Previous studies confirmed that the implanted OCP/collagen focuses and enhances bone regeneration and it is converted to apatite crystals as determined by XRD or Fourier transform infrared spectroscopy.[15,16] The increase of radiopacity in the bone defect originated from apatitic conversion from OCP and bone formation,[15] with most of it being dependent on bone formation.[14,16,21] Therefore, the increased radiopacity in these cases would be involved in new bone formation. This means that the implanted OCP/collagen would enhance bone formation in human bone defects.

The CT values in the defects of both patients were increased from 21 to 23 HU (before implantation) through 327–316 HU (3 months) to 527–602 HU (6 months). As CT value is proportional to bone mineral density, cancellous bone around the bone defect showed a change from ~200 to 600 HU, whereas cortical bone around it changed from ~600 to 1600 HU in the present cases. In addition, that of OCP or OCP/collagen changed from 130 to 140 HU. This suggests that new bone derived from OCP/collagen implantation was almost the same as cancellous bone at 6 months.

At 6 months after OCP/collagen implantation, dental radiography of patient B in the treated defect was more radiopaque than that of patient A, whereas the difference of radiopacity on CT or CT value at the center of the defect was less noticeable. The size of the defect or the amount of the implanted material might be related to the difference between the patients. Although this study remains obscure what would be elicited these difference, it should be examined in the future. Although this study indicated that the implantation of OCP/collagen would regenerate new bone in defects after cystectomy, no comparison of bone regeneration was performed between OCP/collagen-treated cases

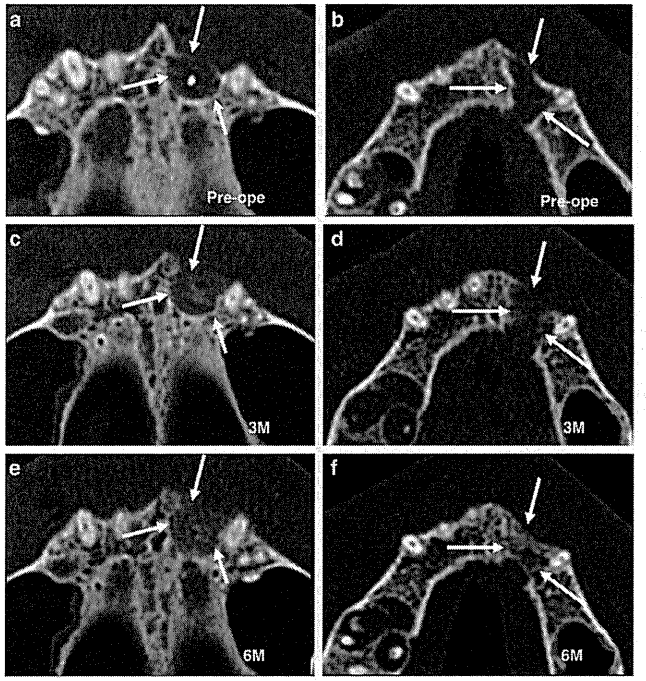

FIG. 6. Computed tomography pictures of horizontal plane. **(a)** Before cystectomy in patient A. The size was almost 8 mm in diameter. **(b)** Before cystectomy in patient B. The size was almost 5×5×10 mm. **(c)** At 3 months after cystectomy in patient A and **(d)** patient B. **(e)** At 6 months after cystectomy in patient A and **(f)** patient B.

and control cases treated with cystectomy but no implantation. This should be considered a limitation of this study, and there is a need for comparative study between OCP/collagen-treated cases and control cases to be performed to clarify the bone regenerative properties of OCP/collagen in the future.

In this study, we confirmed that OCP/collagen disk had good handling performance with softness like that of a sponge and styptic characterization by collagen. Because OCP/collagen itself has little radiopacity, it should be easy for clinicians to confirm bone regeneration in bone defects using X-ray examination. These properties convey some advantages to OCP/collagen in clinical use compared with other bone substitute materials.

TABLE 1. COMPUTED TOMOGRAPHY VALUE AT BONE DEFECT

	Cyst (before cystectomy), HU	*3 months*	*6 months*
Patient A	21	327	527
Patient B	23	316	602

Computed tomography value was measured at the center of the defect.
HU, hounsfield unit.

In this clinical study, OCP/collagen was used in small bone defects created by cystectomy. However, our previous study demonstrated that OCP/collagen implantation enhanced bone healing at tooth extraction socket, alveolar cleft model, and mandibular bone defect in a canine model.[19–21,23] Therefore, OCP/collagen should be able to enhance bone regeneration in large bone defects. Studies to elucidate the bone regenerative properties of OCP/collagen in other bone defects or large bone defects should be performed in humans.

Conclusion

This study demonstrated that OCP/collagen would be safely used and enhanced bone regeneration in human bone defects. To reinforce the efficacy of OCP/collagen as a bone substitute material, it should be compared with other suitable comparators in the future.

Acknowledgments

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Disclosure Statement

The authors (S.K. and O.S.) obtained a patent of OCP/Col in Japan (#5046511).

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Positive auditory cortical responses in patients with absent brainstem response

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HIGHLIGHTS

- N1m response and 40-Hz auditory steady state response could be detected in patients with absent auditory brainstem response.
- N1m response appeared to be more detectable than the 40-Hz auditory steady state response.
- Combined assessment with several different evoked responses may be useful to evaluate the disease state of retrocochlear lesions.

ABSTRACT

Objective: To compare the detectability of the different auditory evoked responses in patients with retrocochlear lesion.
Methods: The 40-Hz auditory steady state response (ASSR) and the N1m auditory cortical response were examined by magnetoencephalography in 4 patients with vestibular schwannoma, in whom the auditory brainstem response (ABR) was absent.
Results: Apparent N1m responses were observed despite total absence of the ABR or absence except for small wave I in all patients, although the latency of N1m was delayed in most patients. On the other hand, clear ASSFs could be observed only in one patient. Very small 40-Hz ASSFs could be detected in 2 patients (amplitude less than 1 fT), but no apparent ASSFs were observed in one patient, in whom maximum speech intelligibility was extremely low and the latency of N1m was most prolonged.
Conclusion: The N1m response and 40-Hz ASSR could be detected in patients with absent ABR, but the N1m response appeared to be more detectable than the 40-Hz ASSR.
Significance: Combined assessment with several different evoked responses may be useful to evaluate the disease conditions of patients with retrocochlear lesions.

1. Introduction

Auditory N1 cortical sensory potentials to tones can often be detected even in the presence of absent or severely deteriorated auditory brainstem response (ABR) (Satya-Murti et al., 1983; Kraus et al., 1993; Starr et al., 1996, 2003, 2004; Rance et al., 2002; Michalewski et al., 2005; Takata et al., 2012). This observation may be superficially somewhat curious, considering that the auditory signals basically run from ear to auditory cortex via the brainstem. However, such phenomena have also been reported in patients with various retrocochlear pathologies such as auditory neuropathy, Friedreich ataxia, spinocerebellar degeneration, and multiple sclerosis (Satya-Murti et al., 1983; Kraus et al., 1993; Starr et al., 1996, 2003, 2004; Rance et al., 2002; Michalewski et al., 2005; Takata et al., 2012). Such cortical responses have not been fully examined in patients with vestibular schwannoma, which is known to be one of the typical retrocochlear lesions associated with deteriorated ABR. More recently, the auditory steady state response (ASSR) modulated at 40 Hz (40-Hz ASSR) has been clinically applied as another type of auditory evoked cortical response in adult patients in the awake condition (Galambos et al., 1981; Picton et al., 2003; Ross et al., 2003; Draganova et al., 2008; Kawase et al., 2012). However, whether 40-Hz ASSR could be obtained in patients with absent ABR has not been reported.

N1m analysis in patients with auditory neuropathy (Takata et al., 2012) showed that extremely delayed N1 response with deteriorated signal to noise ratio could be observed in the retrocochlear lesion. In such cases, N1 measurement with magnetoencephalography (MEG) appears to have an advantage over the usual N1 measurements with electroencephalography, since the auditory evoked N1 responses originating from the primary auditory cortices can be identified by the equivalent current dipole (ECD) locations, which are determined by dipolar source analysis based on MEG with the best fit sphere for each subject's head (Hari et al., 1980; Pantev et al., 1986, 1996; Näätänen and Picton, 1987; Reite et al., 1994; Nakasato et al., 1995, 1997; Kanno et al., 1996, 2000).

The present study used MEG to investigate the occurrence of the 40-Hz ASSR and N1m in vestibular schwannoma patients with absent brainstem response to examine the following three issues: (1) whether the positive N1 cortical response can be detected in vestibular schwannoma patients with absent ABR, as in patients with other types of retrocochlear lesion; (2) whether the 40-Hz ASSR can be detected in patients with absent ABR, in addition to the N1m; (3) if the 40-Hz ASSR can be detected, whether the detectability between N1 response and 40-Hz ASSR is different in patients with deteriorated ABR.

2. Materials and methods

2.1. Patients

This study included 4 patients, 2 males aged 70 and 74 years and 2 females aged 59 and 71 years, with vestibular schwannoma diagnosed in the Department of Otolaryngology-Head and Neck Surgery, Tohoku University Hospital. The patients were recruited based on the following criteria: unilateral or bilateral vestibular schwannoma, severely deteriorated ABR (totally absent or absent except wave I), and threshold at 2 kHz equal to or better than 70 dB HL. In the present study, N1m in response to 2 kHz tone burst at 80 dB SPL and 40-Hz ASSR evoked by 2 kHz AM tone at 80 dB SPL were examined, so the latter criterion was adopted. The present study was approved by the ethical committee of the Tohoku University Graduate School of Medicine and Kohan Hospital. All parts of the present study were performed in accordance with the guidelines of the Declaration of Helsinki.

2.2. Otological examinations

The patients were interviewed to establish any history of hearing loss, followed by ear, nose, and throat examination, then examined by pure-tone audiometry followed by speech audiometry, ABRs, and magnetic resonance (MR) imaging. A list of 20 Japanese monosyllables compiled by the Japan Audiological Society and referred to as List 67-S was used as the stimuli for speech auditory stimuli. The level of speech stimuli using these 20 monosyllables was elevated by 10 dB steps until the maximum percentage of correct answers could be obtained within a sound level not exceeding 100 dB. The order of test stimuli for each sound level was randomly changed.

2.3. ABRs

ABRs were recorded using a silver electrode placed on the mastoid and referred to the vertex. Measurements were conducted in a sound-proof room. Click stimuli were presented through an ear receiver at 10 Hz. Click phases were alternately reversed. The responses to 1000 stimuli were filtered with a band pass filter of 50–3000 Hz, then amplified and averaged using a signal processor (Neuropack S1 MEB 9402, Nihon Kohden, Tokyo, Japan). Severely deteriorated ABRs were determined as totally absent or absent except for wave I responses to clicks at 105 dB.

2.4. Measurement of auditory evoked fields (AEFs)

The stimulus to elicit the N1m response was a tone burst of 60 ms duration (rise and fall times of 10 ms, plateau time of 40 ms) presented monaurally at 2 kHz. The sound pressure level of the tone bursts was 80 dB SPL. The mean interstimulus interval was 3.33 s (0.3 Hz) with 50% interstimulus variance. The stimulus to elicit the ASSR was a 2000-Hz continuous tone 100% AM presented monaurally at 39 Hz with an exponential modulation envelope (John et al., 2002). The sound pressure level of the AM tone was 80 dB SPL. Since the largest amplitude of the cortical ASSR is obtained at just below 40 Hz (Pastor et al., 2002), the modulation frequency of 39 Hz was actually chosen to obtain ASSR as in our previous study (Kawase et al., 2012). The ASSR obtained by modulation around 40 Hz is usually referred to as the "40-Hz ASSR" (Pantev et al., 1996; Ross et al., 2003), so in the present study, the ASSR elicited by the 39 Hz AM tone was also referred to as the "40-Hz ASSR." Both stimuli (AM tone and tone bursts) were presented to the subject through a tube earphone (ER-3A, Etymotic Research, Elk Grove Village, IL; tube length, 1.5 m).

AEFs were recorded in a magnetically shielded room using a 160-channel whole-head type axial gradiometer system (MEGvision PQ1160C, Yokogawa Electric, Musashino, Tokyo, Japan). The first-order axial gradiometers had a baseline of 50 mm with gradiometer coil diameter of 15.5 mm. The uniform gradiometer array was arranged on a helmet-shaped surface at the bottom of the dewar vessel with mean distance of 25 mm between the centers of two adjacent coils. The field sensitivity of the sensors (system noise) was 3 fT/Hz within the frequency range. Subjects were instructed to keep awake during recording, and were assessed by real-time MEG monitoring of the occipital alpha rhythm. If the subjects felt sleepy, wakefulness was restored by conversation and/or by a short nap. The AEFs were recorded only in the awake state.

The N1m responses to tone bursts were obtained from the signals from 100 ms before to 500 ms after the stimulus onset, which were averaged for 300 s (about 100 times). Off-line analysis processed the averaged data with digital band-pass filtering from 3.0 to 40.0 Hz. The N1m response was visually identified as the first prominent peak at longer than 80 ms after the onset, with the isofield map confirming downward current orientation. The 40-Hz ASSR was obtained from the signals after band-pass filtering between 0.03 and 500 Hz and sampling at 2000 Hz. MEG signals were recorded serially for 240 s during the presentation of continuous AM tone, and the responses were analyzed offline. Data epochs of 4-s duration, starting at the onset of the trigger signal synchronized with a certain phase of the amplitude modulation, were extracted from the serial recorded data after digital band-pass filtering (35–45 Hz) and averaged in the time domain. For both the N1m response and auditory steady state field (ASSF), the location of the signal source was estimated using an ECD model with the best-fit sphere for each subject's head. The estimated source was superimposed on the three-dimensional MR image of the individual subject using a MEG–MR image coordination integration

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system, to verify that the measured responses originated from the auditory cortex.

3. Results

Clinical data (MR images, audiograms, and ABRs) in patients with vestibular schwannoma are shown in Fig. 1. Maximum speech intelligibilities are indicated in the lower left of each audiogram. Cases 1, 2, and 4 were unilateral cases, and Case 3 was a bilateral case (neurofibromatosis type 2) with tumors in the bilateral cerebellopontine angles, but apparently deteriorated ABR (absent) was observed only on the left. The ABRs were totally absent in Cases 1 and 3, and were absent except for wave I in Cases 2 and 4.

N1m responses (stacked waveforms), the isofield patterns over the entire head, and the estimated ECDs at peak latency in each hemisphere are superimposed onto the individual horizontal and coronal MR images in Fig. 2. The N1m responses to stimuli of either ear in both the ipsilateral and contralateral hemispheres were compatible with the auditory response, since the source of the N1m was localized in the primary auditory cortex along the superior temporal plane in each hemisphere for either ear stimuli. In all patients, apparent N1m responses were observed despite the totally absent ABR or absent except wave I, although the latency of N1m was delayed in most patients.

Stacked waves of ASSFs, isofield patterns over the entire head, and the estimated ECDs at the amplitude maxima are superimposed onto the individual horizontal and coronal MR images in Fig. 3. ASSFs could be clearly observed in Case 1. Very small 40-Hz ASSFs could be obtained in Cases 2 and 4 (amplitude less than 1 fT), with the estimated ECD located around the auditory cortex. However, no apparent ASSFs were observed in the left ear of Case 3, in which the maximum speech intelligibility was extremely low and the latency of N1m was most prolonged, despite the minimal difference in the auditory thresholds between the ears around the test frequency (2 kHz) of the ASSR and N1m.

In the present cases, wave I of the ABR was preserved in two cases (Cases 2 and 4) and absent in two cases (Cases 1 and 3), so the presence/absence of wave I seemed to have no apparent relationship with the findings of N1m and/or ASSR.

4. Discussion

The present case study showed that, even in patients with absent ABR, positive cortical responses such as the N1m and 40-Hz ASSR could be obtained in the AEFs. The present findings support the idea that positive N1 cortical response can be detected in vestibular schwannoma patients with absent ABR, as in patients with other types of retrocochlear lesion as reported previously (Satya-Murti et al., 1983; Kraus et al., 1993; Starr et al., 1996, 2003, 2004; Rance et al., 2002; Michalewski et al., 2005; Takata et al., 2012), and that 40-Hz ASSR can be detected in patients with absent ABR, in addition to the N1m. Comparison of the abnormalities of the N1m response and 40-Hz ASSR indicated that the N1m response may tend to be more detectable than the 40-Hz ASSR in patients with deteriorated ABR.

Retrocochlear lesions associated with the deterioration of ABR include many different pathologies, but one of the common features may be deterioration of the synchronous neural responses. Absence of the ABR to audible sound is thought to be closely related to such neural dyssynchrony (Satya-Murti et al., 1983; Starr et al., 1991; Berlin et al., 2001, 2003; Takata et al., 2012). For example, deterioration of ABR observed in vestibular schwannoma as in

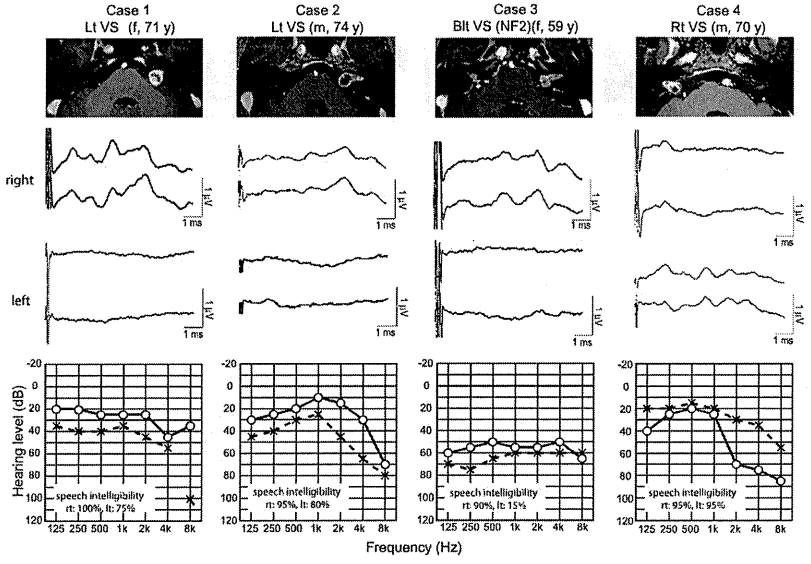

Fig. 1. Clinical findings (MR images, ABRs, and audiograms) in patients with vestibular schwannoma (VS). Maximum speech intelligibilities are indicated in the lower left of each audiogram. ABRs in response to clicks at 105 dB are shown. NF2: neurofibromatosis type 2.

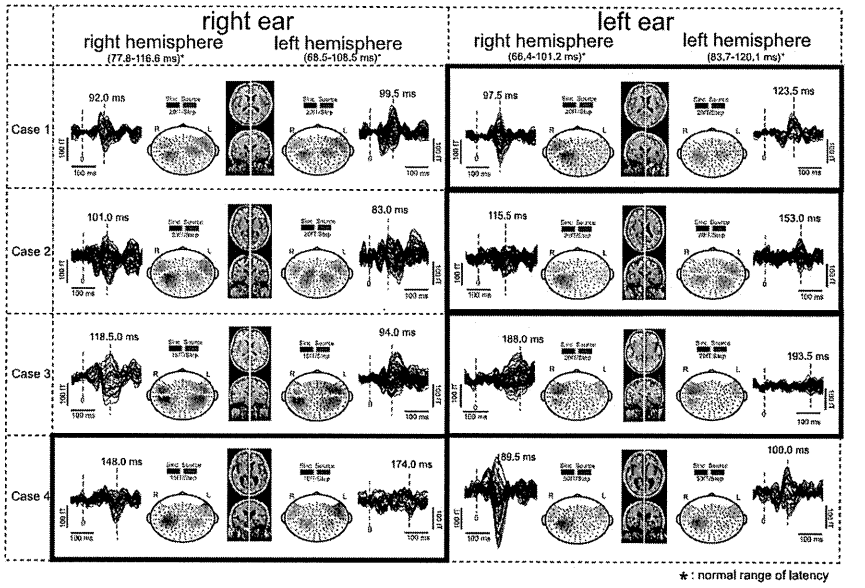

Fig. 2. N1m responses of 4 patients with vestibular schwannoma. Magnetic signals (stacked waveforms) from 50 ms before to 350 ms after the stimulus onset in each hemisphere in response to left ear and right ear stimulation, in addition to the isofield patterns over the entire head and the estimated ECDs at the peak latency in each hemisphere, are superimposed onto the individual horizontal and coronal MR images. Data obtained from stimulation to the ipsilateral ear to the tumor are surrounded with thick lines.

the present cases is thought to be due to the neural dyssynchrony caused by the "neural conduction block" resulting from compression by the tumor (Ylikoski et al., 1978; Kveton, 1990; Kaga et al., 1997; Roberson et al., 1999). On the other hand, deterioration in ABR in patients with auditory neuropathy may result from pathological dysfunction of the synapses between the inner hair cells and the auditory neurons or demyelinization of the auditory nerve (Starr et al., 1991; Berlin et al., 2001, 2003). Therefore, in any case, such pathologies presumably result in the deterioration of synchronization of spike timing between neurons, resulting in severe effects on the wave formation of the ABR, which is the sum of the synchronized spike activities of the neurons evoked by sound stimuli. Therefore, it may be reasonable to speculate that the positive auditory cortical response in patients with absent brainstem responses may reflect varying fragility to dyssynchrony of the neural responses.

The different susceptibilities to neural dyssynchrony of the ABR, 40-Hz ASSR, and N1m may be partly explained by the different configurations of the waves of these three responses. Different configurations of the response waves between different evoked responses may reflect different susceptibilities to neural dyssynchrony of the ABR (Takata et al., 2012). That is, the durations between the onset and the endpoint of the waves of the ABR were much shorter (about 1/100) than that of the N1m response (N1m: 63.0 ± 11.3 ms [n = 20], wave 1 of ABR: 0.58 ± 0.1 ms [n = 20], wave V of ABR: 0.89 ± 0.2 ms [n = 20]), and as a result, the effects of "dyssynchrony" might be much larger on the ABR than on the N100m (Takata et al., 2012). On the other hand, the similar parameter of the 40-Hz ASSR (i.e., one phase of the 40-Hz sine wave) appears to be 25 ms. Based on the present hypothetical considerations, the fragility of the 40-Hz ASSR to dyssynchrony would occur between those of N1 and ABR.

In the present clinical study, the N1m in response to 2 kHz tone bursts at 80 dB SPL was observed in all 4 patients. In contrast, the 40-Hz ASSF elicited by 2 kHz AM (modulated with 40 Hz) tone at 80 dB SPL could be clearly observed in only one patient (Case 1), in whom the deterioration of the N1 response was most minimal (latency of N1 was nearly normal even in the pathological side); i.e., although the number of subjects examined in the present study was small, the clinical findings appear to be consistent with the above-mentioned hypothesis. Moreover, based on the proposed hypothesis, the ASSR to different modulation frequencies may have different fragilities to dyssynchrony of the neural response, although only the 40-Hz ASSR was examined in the present study. ASSRs using several different modulation frequencies might be a better objective procedure for the assessment of hearing loss caused by retrocochlear lesion.

Previously, we have considered the different susceptibilities of the different evoked responses based on the very simplified concept of "neural synchrony". However, the generator site and mechanism of each response are very different for the different responses, so the effects of supposed dyssynchrony occurring in the peripheral neurons may not be so simple.

One of the psychophysical characteristics of hearing loss caused by retrocochlear lesion is the greater deterioration in speech intelligibility than expected based on the findings of pure-tone audiog-

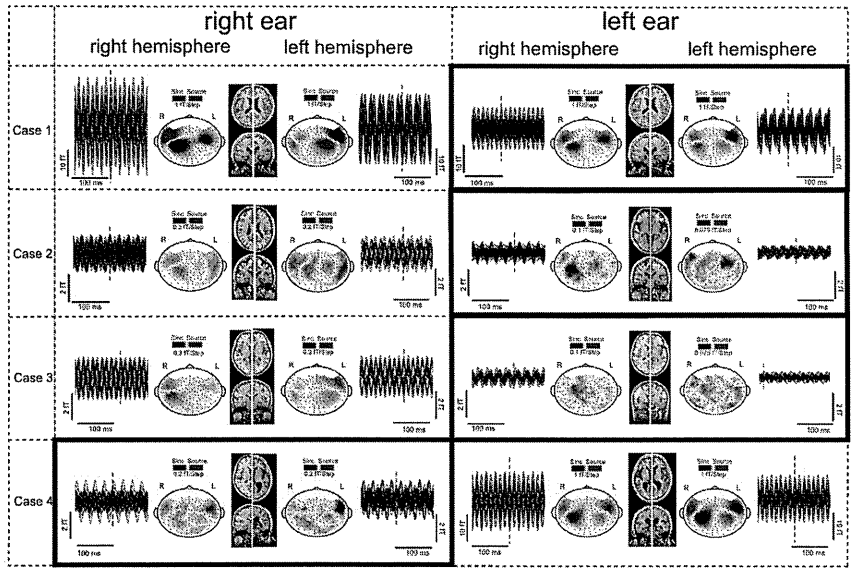

Fig. 3. 40-Hz ASSFs of 4 patients with vestibular schwannoma. Magnetic signals for 200 ms (stacked waveforms) in each hemisphere in response to left ear and right ear stimulation, in addition to the isofield patterns over the entire head and the estimated ECDs at the peak latency in each hemisphere, are superimposed onto the individual horizontal and coronal MR images. Data obtained from stimulation to the ipsilateral ear to the tumor are surrounded with thick lines.

raphy (Starr et al., 1996). A positive relationship has been reported between the presence or absence of cortical N1 responses and speech intelligibility in young children with auditory neuropathy (Rance et al., 2002). In the present study, the worst deteriorations of cortical responses (absence of 40-Hz ASSR and extreme prolongation of N1m responses) were observed in the patient with the worst speech intelligibility. Despite the limited number of the subjects, the present results seem to be consistent with the previously reported findings. As for the relationship between speech intelligibility and evoked responses, the present study suggests that the combination of different types of evoked response including the ASSR may be better objective tools to assess the degree of deterioration of speech intelligibility, which is assumed to be related to neural dyssynchrony. This aspect should be further examined in a larger number of cases using not only the N1m and 40-Hz ASSF, but also ASSFs in response to different several modulation frequencies.

Combined assessment with several different evoked responses may be also useful for the preoperative investigation of patients with vestibular schwannoma. Preoperative prediction of possible hearing preservation is one of the important factors in the decision-making process concerning the surgical removal of vestibular schwannoma. ABR has been most frequently used for the preoperative assessment of vestibular schwannoma among the auditory evoked responses (Glasscock et al., 1987; Ogawa et al., 1991; Nadol et al., 1992; Matthies and Samii, 1997; Roberson et al., 1999). Generally, the presence of ABR waves is thought to be a positive indicator for postoperative hearing preservation (Glasscock et al., 1987; Matthies and Samii, 1997), whereas the absence of ABR does not necessarily indicate no chance of postoperative hearing preservation (Roberson et al., 1999). In this respect, measurements of the N1m and ASSRs in addition to the ABR may provide additional important information. Therefore, combined assessment with several different evoked responses may be one of the most useful methods to evaluate the disease conditions of patients with retrocochlear lesions, especially the state of deterioration of synchronous neural response.

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Review Article

Rehabilitation with Poststroke Motor Recovery: A Review with a Focus on Neural Plasticity

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Motor recovery after stroke is related to neural plasticity, which involves developing new neuronal interconnections, acquiring new functions, and compensating for impairment. However, neural plasticity is impaired in the stroke-affected hemisphere. Therefore, it is important that motor recovery therapies facilitate neural plasticity to compensate for functional loss. Stroke rehabilitation programs should include meaningful, repetitive, intensive, and task-specific movement training in an enriched environment to promote neural plasticity and motor recovery. Various novel stroke rehabilitation techniques for motor recovery have been developed based on basic science and clinical studies of neural plasticity. However, the effectiveness of rehabilitative interventions among patients with stroke varies widely because the mechanisms underlying motor recovery are heterogeneous. Neurophysiological and neuroimaging studies have been developed to evaluate the heterogeneity of mechanisms underlying motor recovery for effective rehabilitation interventions after stroke. Here, we review novel stroke rehabilitation techniques associated with neural plasticity and discuss individualized strategies to identify appropriate therapeutic goals, prevent maladaptive plasticity, and maximize functional gain in patients with stroke.

1. Introduction

Despite advances in acute management, stroke remains a major cause of disability worldwide [1–6]. A number of neurological functions are impaired by stroke, the most common of which is motor disability contralateral to the stroke lesion side [7]. Therefore, many rehabilitation techniques based on motor learning paradigms have been developed to facilitate the recovery of impaired movement in patients with stroke [3, 8–11].

Neural plasticity can change central nervous system structure and/or function [12–15]. Recently, advances in technologies enabling noninvasive exploration of the human brain have increased our understanding of neural plasticity and its relationship to stroke recovery [9, 12, 16, 17]. Various novel stroke rehabilitative methods for motor recovery have been developed based on basic science and clinical studies characterizing brain remodeling due to neural plasticity [9, 11, 18]. The effectiveness of these approaches has been verified by systematic reviews and meta-analysis studies [8, 19–22]. However, responses to rehabilitative interventions show large inter-individual variation because the mechanisms underlying motor recovery are heterogeneous across patients [3, 8, 11, 23]. Furthermore, these mechanisms involve complex processes including restitution, substitution, and compensation that rely on a combination of spontaneous and learning-dependent processes [3, 24]. Therefore, elucidating the mechanisms underlying motor recovery can help to identify the most appropriate type, duration, and goals of individual rehabilitation strategies after stroke [11]. Neurophysiological and neuroimaging approaches have recently been developed to evaluate the heterogeneity of motor recovery mechanisms to better understand and predict the effectiveness of different rehabilitation interventions after stroke [12, 16, 25, 26].

In this review, we first discuss the principles of stroke rehabilitation in task-specific training and enriched environments. Then, we focus on novel strategies in stroke

rehabilitation that are supported by evidence of associated neural plasticity. These approaches include constraint-induced movement therapy (CIMT), body weight-supported treadmill training (BWSTT), robotic training, transcutaneous neuromuscular electrical stimulation, noninvasive brain stimulation (NIBS), action observation, virtual reality (VR) training, and brain-computer interface (BCI). Finally, we discuss individualized strategies to inform the identification of therapeutic goals, to prevent maladaptive plasticity, and to maximize functional gain in patients with stroke.

2. Principles of Stroke Rehabilitation

Most protocols for stroke rehabilitation are based on motor learning, which induce dendrite sprouting, new synapse formation, alterations in existing synapses, and neurochemical production [10, 27]. These changes are thought to provide a mechanistic substrate to facilitate motor recovery after stroke [10, 27]. Motor learning is known to be greater if the practice method is meaningful, repetitive, and intensive [10, 17]. Further, it is recommended that stroke rehabilitation is applied in stroke care units where multidisciplinary teams can support active patient participation [9]. In this section, we review task-specific training and enriched environment therapeutic approaches that facilitate neural plasticity.

2.1. Task-Specific Training. Motor training after stroke should be targeted to goals that are relevant to the functional needs of the patient [10, 11]. Therefore, focusing on task-specific training to facilitate activities of daily living or other relevant motor tasks is a well-accepted principle of stroke rehabilitation [3]. This approach has been described by a variety of terms, including repetitive task practice, repetitive functional task practice, and task-oriented therapy [10, 28, 29]. Thus, task-specific training emphasizes the repetitive practice of skilled motor performance to improve individual functional abilities [10, 30]. Task-specific training can effectively recover a wide array of motor behaviors involving the upper limbs, lower limbs, sit-to-stand movements, and gait after stroke [29, 31–33]. Furthermore, repetitive task-specific training has been found to achieve better functional gains compared to nonrepetitive training [34, 35].

Increasing evidence suggests the involvement of neural plasticity in task-specific training [36, 37]. A meta-analysis of neurophysiological and neuroimaging studies has reported that neural changes in the sensorimotor cortex of the affected hemisphere accompany the gains in functional paretic upper extremity movements achieved with task-specific training [37]. Compared to traditional stroke rehabilitation approaches such as simple motor exercises, task-specific training induces long-lasting motor learning and associated cortical reorganization [30, 37]. Thus, there is strong evidence demonstrating that task-specific training can assist with functional motor recovery, which is driven by adaptive neural plasticity [8, 24, 30, 37, 38].

2.2. Enriched Environment. In addition to task specificity, the therapeutic environment plays an important role in stroke rehabilitation [39]. Environments that provide greater opportunity for physical activity and motivation are referred to as enriched environments [39]. Animal studies involving rat models of stroke have demonstrated that enriched environments facilitate motor recovery and neural plasticity because they present greater opportunities for physical activity, play, and social interactions compared to standard laboratory cages [39–41].

Clinically, stroke unit (SU) care administered by a well-coordinated multidisciplinary team can provide an enriched environment for patients with stroke [42]. SU care provides an organized package of care through a cyclical process involving the necessary elements of assessment, goal setting, intervention, and reassessment [3, 11]. Moreover, SU care provides individuals with a clear understanding of what is expected of them during task-specific training, resulting in neural plasticity that improves their performance [43]. Patient involvement in patient-centered interdisciplinary goal setting has been shown to encourage their motivation and engagement in therapy, resulting in better rehabilitation outcomes of impaired movement in patients with stroke [3]. Several studies have demonstrated that SU care had the greatest positive impact on disability levels after stroke [42, 44]. Moreover, the reported benefits of SU care extend to patients of all ages and to patients with varying stroke severity [44]. Thus, stroke rehabilitation programs should include meaningful, repetitive, intensive, and task-specific movement training in an enriched environment in order to promote neural plasticity and motor and functional recovery [10, 17].

3. Novel Strategies Based on Motor Training

During the last several decades, many studies have reported the use of novel motor learning-based stroke rehabilitation strategies [3, 8–11]. In this section focused on neural plasticity, we discuss several representative neurorehabilitation methods, including CIMT, BWSTT, and robot training.

3.1. CIMT. Patients with stroke often use the nonparetic limb instead of the paretic limb to perform daily activities. Dominant use of the nonparetic limb induces the phenomenon of learned nonuse in the paretic limb, which limits the capacity for subsequent gains in motor function [38, 45]. CIMT is a therapeutic strategy that was developed to overcome learned nonuse of the paretic limb. It forces paretic arm use by requiring a patient to perform functionally oriented activities while the nonparetic arm is physically restrained with a sling or glove. Mechanistically, the repetitive training of the paretic arm and constraint of the nonparetic upper arm used in CIMT might both be important for promoting neural plasticity. Skill acquisition with the nonparetic limb has been reported to negatively impact the use-dependent plasticity of the affected hemisphere in animal models of stroke [46]. The reasons underlying this constraint remain unclear, but this phenomenon may reflect use-dependent alterations in interhemispheric connectivity [47, 48]. Therefore, constraint of the nonparetic limb itself might ameliorate the impairment of use-dependent plasticity of the paretic limb after stroke

[15, 45]. Several studies reported neural plasticity after CIMT as evidenced by neuroimaging and neurophysiological techniques [49–51]. Previous studies using transcranial magnetic stimulation (TMS) found that the cortical representation size of the paretic hand was increased after therapy [49, 50]. Neuroimaging studies also demonstrated altered neural network activity after CIMT [49, 51]. Moreover, a structural magnetic resonance imaging (MRI) study reported that CIMT increased gray matter in the bilateral sensorimotor cortices compared with control therapy [52]. Thus, there is evidence that CIMT induces both structural brain and physiological changes in patients with stroke [10].

Wolf et al. conducted a multicenter single-blind randomized controlled trial known as the Extremity Constraint-Induced Therapy Evaluation Trial to compare the effects of 2-week CIMT with customary care in 222 individuals within 3–9 months of a first stroke [53]. At 1 year, the CIMT group performed better on functional tasks using the paretic upper limb. Moreover, the 2-year follow-up documented no decline from the 1-year assessment, and there were trends toward continued improvement of strength during the second year [54]. Most reviews of CIMT also report trends towards positive results of motor recovery in patients with chronic stroke [8–10]. However, previous studies had reported no significant differences in motor recovery between CIMT and an equal dose of traditional therapy for patients with acute stroke [55, 56]. This could be due to minimal or no learned nonuse during the acute phase [10]. Moreover, in the acute stage of stroke, high-intensity CIMT results in less improvement than low-intensity CIMT [56]. Therefore, additional studies are needed to explore optimal CIMT timing and intensity for motor recovery after stroke [11].

3.2. BWSTT. BWSTT is a rehabilitation method in which patients with stroke walk on a treadmill with their body weight partially supported. BWSTT augments the ability to walk by enabling repetitive practice of complex gait cycles [57, 58]. In patients who have experienced a stroke, hemiparesis can cause abnormal control of the paretic lower limb, resulting in an asymmetrical gait pattern [59, 60]. Partial unloading of the lower extremities by the body weight support system results in straighter trunk and knee alignment during the loading phase of walking [61, 62]. BWSTT also improves swing time asymmetry, stride length, and walking speed [60, 62, 63]. Therefore, BWSTT allows the patient to practice nearly normal gait patterns and avoid developing compensatory walking habits, such as hip hiking and circumduction [58, 64].

There is evidence of gait improvement after BWSTT, including use of robotic device systems, compared to conventional therapy in patients with acute stroke and those with chronic stroke [60, 65, 66]. However, a recent study reported that the benefits of BWSTT were not superior to that achieved with home-based physical therapy that emphasized strength and balance, regardless of whether BWSTT was started 2 or 6 months after the stroke [67]. Moreover, among patients with severe walking impairments, multiple falls were more common in the group that received early BWSTT compared to the group that received late BWSTT and physical therapy [67]. Therefore, BWSTT programs should include balance training that helps prevent falls in patients, especially those with acute stroke and severe impairment.

Mechanistically, BWSTT is believed to increase brain activity in the bilateral primary sensorimotor cortices, cingulate motor areas, caudate nuclei, and thalamus of the affected hemisphere [68]. Moreover, BWSTT has been found to alter central pattern generator activation in animal studies [69, 70]. In patients who have experienced a stroke, cerebral cortex function is impaired while that of the spinal cord is preserved. However, spinal cord changes may also be important for gait recovery after stroke due to changes in signals received following cerebral reorganization [71]. Thus, BWSTT can be used in patients with stroke to induce reorganization at the spinal and supraspinal levels, reduce gait parameter asymmetries, and increase walking speed. However, evidence of neural plasticity involved in this process is restricted to animal studies [71].

3.3. Robot Training. Robotic training offers several potential advantages in stroke rehabilitation, including good repeatability, precisely controllable assistance or resistance during movements, and objective and quantifiable measures of subject performance [72]. Moreover, robot training can provide the intensive and task-oriented type of training that has proven effective for promoting motor learning [8, 72]. These characteristics of robot training are thought to be useful for motor recovery after stroke.

During the last decades, mechanically assisted robot training therapies have been developed for stroke rehabilitation to improve arm function [21, 73–75]. However, a multicenter, randomized controlled trial of patients with chronic stroke who had moderate-to-severe upper-limb impairment reported no difference in motor recovery between intensive physiotherapy and robot-assisted rehabilitative therapy [76]. Moreover, systematic reviews and meta-analyses have found no significant changes in activities of daily living ability after robotic training [77, 78]. Automated electromechanical gait machines have also been developed to facilitate lower limb rehabilitation. These machines consist of either a robot-driven exoskeleton orthosis or 2 electromechanical footplates that simulate gait phases [79–81]. Such machines are useful because they do not require therapists to set the paretic limbs and control weight shift, as is required for treadmill training [79, 80]. The use of electromechanical-assisted gait-training devices in combination with physiotherapy increases the chance of regaining independent walking ability after stroke but does not produce improvements in walking speed [82]. Therefore, in addition to automatic repetitive motor training, it is important for augmentation of robot training that robotic assistance is carried out in a minimum difference of input-output timing using electromyography (EMG) and/or position feedback [75, 83, 84]. Reducing these lag times is important because synchronization between sensory and motor information facilitates neural plasticity [85, 86]. Future studies are needed to determine the most appropriate characteristics of subjects and whether robot training has advantages over conventional therapy [75].

4. Augmentation of Use-Dependent Plasticity

Although the use-dependent plasticity induced by motor training is important for motor recovery after stroke, it has been reported that use-dependent plasticity is impaired in the affected hemisphere [87, 88]. Therefore, it is important to augment neural plasticity after stroke to facilitate motor recovery. In this section, we discuss the following possible methods of augmenting use-dependent plasticity in patients with stroke: transcutaneous neuromuscular electrical stimulation and NIBS.

4.1. Transcutaneous Neuromuscular Electrical Stimulation. Transcutaneous neuromuscular electrical stimulation can improve neuromuscular function in patients with stroke by strengthening muscles, increasing motor control, reducing spasticity, decreasing pain, and increasing range of motion [89]. Methods of transcutaneous neuromuscular electrical stimulation are generally categorized as either therapeutic electrical stimulation or functional electrical stimulation (FES). The defining feature of FES is that it provokes muscle contraction and produces a functionally useful movement during stimulation [89]. Several upper extremity FES devices are available, and the use of these devices seems to have a positive effect on upper-limb motor function in both acute and chronic stages of stroke [90–92]. FES has also been combined with different walking training strategies and has been shown to result in improvements in hemiplegic gait in both acute and chronic stages of stroke [93–95].

In addition to functional effects, FES is thought to have therapeutic effects, which are postulated to arise through the facilitation of neural plasticity by increasing the strength of afferent inputs [89]. In particular, FES supported by an EMG- or position-triggered system could induce appropriate proprioceptive feedback and promote motor learning [89, 96]. Patients can actively participate in intensive and repetitive task-specific training when they are responsible for initiating practice. Moreover, the synchronization of afferent feedback with voluntary movement by a biological signal-triggered system is useful for motor recovery because synchronization between the sensory and motor information facilitates neural plasticity [85, 86]. In fact, better performance is observed if paretic muscles are stimulated by voluntary muscular activity compared with nonsynchronized passive stimulation [97]. However, future research is needed to determine the most effective type and dose of electrical stimulation [98].

4.2. NIBS. Repetitive TMS (rTMS) and transcranial direct current stimulation (tDCS) are NIBS techniques that can alter human cortex excitability [99]. NIBS therapy for motor recovery following stroke aims to augment neural plasticity and improve motor function based on the interhemispheric competition model, which proposes that motor deficits in patients with stroke are due to reduced output from the affected hemisphere and excessive interhemispheric inhibition from the unaffected hemisphere to the affected hemisphere [18, 100, 101]. Therefore, NIBS achieves improvement in motor deficits by either increasing the excitability of the affected hemisphere or decreasing the excitability of the unaffected hemisphere [18, 102, 103]. Inhibitory NIBS increases excitability in the ipsilesional motor cortex by reducing excessive interhemispheric inhibition from the contralesional motor cortex [101, 104, 105]. Excitatory NIBS over the affected hemisphere directly increases the excitability of the ipsilesional motor cortex [105–108]. Motor cortex excitability enhancement appears to be required for motor learning [109, 110]. In fact, pairing of rehabilitative training with NIBS results in more enduring performance improvements and functional plasticity in the affected hemisphere compared with motor training or stimulation alone in patients with chronic stroke [101–104, 111]. Furthermore, cumulative NIBS has been shown to be important for continuous motor improvement in patients with stroke [112, 113]. This result indicates that neural plasticity is consolidated by cumulative NIBS intervention. Therefore, NIBS induces a more suitable environment for neural plasticity by artificially modulating the ipsilesional motor cortex, thus counteracting use-dependent plasticity impairment by facilitating plasticity in the affected hemisphere [18].

The effectiveness of NIBS is not limited to the chronic stage; it has been reported that both inhibitory and excitatory NIBS facilitate motor recovery in patients with stroke at the acute stage [108, 114–116]. However, another study reported that inhibitory and excitatory NIBS does not facilitate motor recovery in patients in acute stages of stroke [117, 118]. These discrepant findings underscore the importance of identifying the more effective type of NIBS, as well as optimal timing after stroke. A recent meta-analysis study of rTMS on upper-limb motor function in patients with stroke reported that inhibitory rTMS over the unaffected hemisphere might be more beneficial than excitatory rTMS over the affected hemisphere [22]. Although additional research has begun to evaluate the effectiveness of different NIBS stimulation protocols for motor recovery after stroke, further well-designed studies in larger populations are required to determine whether NIBS in the acute stroke stage can improve motor function and to identify the most effective NIBS protocols, including tDCS for stroke treatment [18].

5. Integration between Motor Learning and Multisensory Feedback

Multisensory feedback plays an important role in motor learning by reestablishing the sensorimotor loop that is disrupted by stroke [9]. Several multisensory feedback approaches have been reported for motor recovery in patients with stroke, including action observation and VR training [19, 119]. Recently developed BCI technology might also facilitate motor recovery by using robot devices and/or electrical stimulation [120].

5.1. Action Observation. There is increasing experimental evidence that some motor neural structures are recruited not only when actions are actually executed but also when the actions of another person are simply observed [121]. The neurophysiological basis for this recruitment is associated with mirror neurons, which have been identified in nonhuman primates [122, 123]. Human studies have also described a "mirror neuron system" involved in action understanding, imitation, motor learning, and modulating training effects [124–127]. According to the mirror neuron paradigm, action observation appears to activate the motor system similar to execution by generating an internal representation of action that can be targeted for motor learning [128–130]. A previous study in healthy subjects reported that observing another person learn a novel task improves subsequent performance of the same task [126]. Moreover, data from a recent virtual lesion study using TMS further supports the hypothesis that action observation coupled with physical practice may enhance use-dependent plasticity through the mirror neuron system in healthy controls [131].

Several clinical studies have reported that a combination of action observation therapy and physiotherapy improve upper-limb motor function in patients with chronic stroke [132, 133]. A recent multi-center randomized control trial demonstrated that action observation with physiotherapy has a positive effect on motor recovery in the acute stage of stroke [134]. Another study that employed functional MRI (fMRI) found that action observation facilitated motor recovery after stroke by reactivating the neural circuit containing the action observation/action execution matching system, which includes the bilateral ventral premotor cortex, supplementary motor area, and the contralateral supramarginal gyrus [132]. Therefore, increased activation of these areas suggests that the mirror neuron system (or its human homolog) may play an important role in motor learning and recovery related to action observation in patients with stroke [132, 134]. Moreover, action observation is safe and can be repetitively conducted without dependency on residual motor function. Despite the increasing evidence that action observation may become a useful strategy in stroke rehabilitation, future research is required to determine optimal practice intensity and duration before its translation into standard clinical practice [119].

5.2. VR. VR is a computer-based technology that engages users in multisensory simulated environments, including real-time feedback (e.g., visual, auditory, and tactile feedback), allowing users to experience simulated real-world objects and events [135]. VR applications range from nonimmersive to fully immersive depending on the degree to which the user is isolated from the real surroundings when interacting with the virtual environment [136]. Immersive VR systems use large-screen projections, head-mounted displays, cave systems, or videocapture systems to immerse the user in a virtual environment [136]. In contrast, nonimmersive VR systems simply use a computer screen to simulate an experience with or without interface devices, such as a computer mouse, joystick, or force sensation [136]. VR exercise applications can easily provide patients with stroke with repetitive, intensive, and task-specific training and can apply relevant concepts for driving neural plasticity that produce motor function improvements after stroke [8, 136, 137]. Several studies have shown that the use of immersive VR results in practice-dependent enhancement of the affected arm by facilitating cortical reorganization [138, 139]. Moreover, a recent study has shown that video game applications that are classified as nonimmersive VR systems can be combined with conventional rehabilitation for upper arm improvement after stroke [140]. Video game systems have already been developed for home use, making this technology less costly and more accessible to clinicians and individuals [137]. Moreover, VR-based game systems can easily adjust task difficulty according to user capability [141, 142]. This encourages the user to train at optimal-level errors, inducing appropriate motivation and arousal, which are important for learning [143]. Therefore, VR-based game systems might be able to facilitate motor learning due to increased motivation of patients with stroke. However, the use of VR is not yet commonplace in clinical rehabilitation settings; only a few studies have been conducted, and the sample sizes were too small to draw firm conclusions [19].

5.3. BCI. BCI systems record, decode, and translate measurable neurophysiological signals into effector actions or behaviors without the use of peripheral physiological activities [144]. Several methods are available for detecting and measuring brain signals, including electroencephalography, electrocorticography, intracortical recordings, magnetoencephalography, fMRI, and functional near-infrared spectroscopy [144, 145]. One of the most popular neurophysiological phenomena assessed in BCI research is the modulation of sensorimotor rhythms through motor imagery [120, 144, 145]. The output of the BCI provides multisensory feedback to users, and this allows them to modulate their brain activity accordingly [144]. The feedback consists of sensory stimuli, such as visual, auditory, or tactile stimuli, and kinesthesia by robotic devices or FES [120, 145]. Therefore, BCI devices can couple intention with action and enable patients with stroke to achieve intended motor action [120, 144]. Considering that BCI technology is based on feedback and exploits learning mechanisms, BCI technology could be used to design and develop specific neurorehabilitation therapies for patients with stroke [120]. In fact, a recent study that combined motor training and motor imagery-based BCI reported a positive trend of upper-limb movement control in patients with stroke [146, 147]. BCI systems also might be useful for patients with severe stroke because they provide an alternative way of executing motor outputs through robotic devices [120, 144, 145]. Moreover, invasive BCI systems that utilize an intracortical recording technique have been developed in animal studies; these systems can detect signals, including synaptic and neuronal activities, and might facilitate neural plasticity due to accurate matching between motor intention and sensory feedback [145, 148–150]. However, the number of studies evaluating stroke recovery after BCI training is still limited. Future studies must evaluate the effect of BCI use on motor recovery after stroke and the role of BCI in neural plasticity [120].

6. Potential Individualized Rehabilitation Strategies for Appropriate Reorganization

An accurate prognosis of motor recovery after stroke can help to select individual rehabilitation strategies that promote appropriate reorganization [11]. It also is important for rehabilitation strategies to prevent maladaptive plasticity, which weakens motor function and limits motor recovery [15]. In this section, we discuss several potential individualized rehabilitation strategies to inform therapeutic goal setting, prevent maladaptive plasticity, and maximize functional gains in patients with stroke.

6.1. Imaging and Neurophysiological Findings Predict Motor Recovery. The simplest indicator of prognosis for patients with stroke is the degree of motor impairment. Many studies have suggested that motor outcomes are positively correlated with initial motor impairment after stroke [151–153]. However, the patterns of motor recovery are largely heterogeneous among patients with stroke; accurate prediction based on current motor impairment status alone can be difficult [11, 25]. Therefore, it has been suggested that motor recovery after stroke may be predicted more accurately using neurophysiological and neuroimaging findings [16, 25, 154]. Neurophysiological studies using TMS have revealed that ipsilesional corticospinal motor projection function is a good predictor of motor outcome after stroke [16, 25]. Neuroimaging studies using diffusion tensor imaging also have revealed that impairment of the ipsilesional corticospinal motor projections could predict motor recovery after stroke [25, 154]. Moreover, evaluation of the ipsilesional corticospinal tract function might facilitate the selection of rehabilitation strategies based on the prediction of potential functional gain, which is an individual's capacity for further functional improvement during the chronic stage of stroke recovery [25]. A recent study reported that the extent of injury to motor projections from supplementary and premotor areas of the affected hemisphere is also useful for predicting potential functional gains of paretic upper limbs from robot therapy in subjects with chronic stroke [26]. These results suggest that measures of motor tract function could be useful in estimating potential motor recovery of patients with stroke entering experimental neurorehabilitation trials and for patient selection in clinical trials [26]. Conversely, other studies have reported that the degree of ipsilesional corticospinal tract damage is not strongly associated with walking function [63, 155]. Moreover, the extent of lesion overlap with the corticospinal motor projections is only weakly correlated with therapy-related gains of gait function [63]. These findings support the importance of subcortical control, including the spinal cord, for lower-limb movements such as walking [156, 157]. Moreover, there is some evidence that ipsilateral motor projections are important in the recovery of walking function [158]. Therefore, the ipsilesional corticospinal motor projections appear to be less important in the control of walking than in the control of upper-limb dexterity after stroke [63].

In addition to motor tract function, identifying individual pattern of cortical activation may predict the effect of rehabilitation technique for patients with motor stroke. An fMRI study reported that lower baseline activity of the ipsilesional motor cortex during paretic hand movement was associated with greater functional gains after 6 weeks of rehabilitation therapy in chronic patients [159]. This result indicates that low baseline cortical activity might represent underuse of surviving cortical resources and possible responsiveness to rehabilitation therapy [159, 160]. Therefore, the motor projections may set a limit on the extent of recovery, but other parameters (e.g., preserved cortical activity) might be important when considering whether a patient has the capacity or potential to improve [160]. However, predicting functional gains by using individual cortical activity patterns may be more difficult than that by utilizing motor tract function. For example, a previous study reported that ipsilesional motor cortex excitability in good responders with chronic subcortical stroke for excitatory rTMS over the affected hemisphere is strongly activated, but not weakly, when moving the paretic hand before rTMS [161]. Moreover, functional gain has no direct correlation with ipsilesional motor cortex activity in the acute stage of stroke, but a pattern of cortical activation including the postcentral gyrus and cingulate cortex correlates with subsequent motor recovery [162]. Furthermore, in patients with stroke and severe initial hemiparesis, subsequent motor recovery was not predicted by task-related fMRI activation [163]. Thus, the effective neural activation pattern for neurorehabilitation might be different depending on time since stroke, lesion site, impairment of motor function, and/or rehabilitation technique due to the heterogeneous mechanisms underlying motor recovery and neurorehabilitation techniques.

Genetic factors of neural plasticity-related components should also be considered to affect the capacity of an individual patient's brain to recover motor function [164, 165]. Moreover, genetic variation might be able to explain some of the variability encountered in motor rehabilitation efficacy [23, 165, 166]. It has been reported that various genetic factors influence neural plasticity in animals and humans (for review see [165]). However, there is no evidence regarding individualized rehabilitation strategies using genetic information.

6.2. Preventing Maladaptive Plasticity. Although some neural plasticity undoubtedly contributes to motor recovery after stroke, it remains unclear whether all forms of neural plasticity contribute to genuine motor recovery [12, 14, 167]. Maladaptive plasticity that weakens motor function and limits recovery has recently been reported after stroke [15, 46, 48, 100, 168]. Therefore, it is important for individual stroke rehabilitation strategies to prevent maladaptive plasticity.

Several studies have suggested that neural plasticity associated with compensatory movement might contribute to maladaptive plasticity after stroke [15, 38]. To perform daily tasks, patients with stroke often develop a compensatory hyperreliance on the nonparetic side, proximal paretic side,

or trunk movement [169–172]. However, this strong and efficient motor compensation may prevent the affected side from generating normal motor patterns for daily activities [38, 169]. In particular, dominant use of the nonparetic limb induces learned nonuse of the paretic limb and limits its functional improvement [38, 45]. The facilitation of neural plasticity underlying compensatory learning with the nonparetic limb after stroke also exacerbates use-dependent plasticity impairment of the affected hemisphere via abnormal interhemispheric inhibition [47, 48]. CIMT that combines a rehabilitative training regime for the paretic limb with constraint of the nonparetic limb can overcome learned nonuse of the paretic limb and has been shown to improve motor function in patients with stroke [45, 53, 173]. Therefore, clinicians should consider CIMT for patients having stroke who fit its criteria to facilitate appropriate reorganization and prevent maladaptive plasticity. However, patients with stroke and severe motor function impairments are not suitable candidates for CIMT therapy. Studies of animal stroke models suggest that compensatory use of the nonparetic limb while the paretic limb is being used does not necessarily result in learned nonuse [46]. Therefore, patients with stroke and poor motor function who engage in compensatory use of the nonparetic limb in daily activities may benefit from bilateral movement training to prevent learned nonuse of the paretic side [15, 25].

Increased activity of the paretic proximal arm due to compensatory movement may contribute to the abnormal interjoint movement in the proximal limb that is, often observed after a stroke [172]. Therefore, the selected rehabilitation program may have to avoid intense training of the paretic proximal side. To our knowledge, no rehabilitation program currently addresses this problem, and compensatory movement of the paretic proximal muscle is useful for reaching in some patients with stroke and poor motor function [38, 174]. Thus, at least in cases where patients with stroke have good motor function, a rehabilitation program that avoids compensatory use of the paretic proximal side may be helpful.

7. Conclusion

Most stroke rehabilitation protocols are based on motor learning to induce neural plasticity, which refers to the ability of the brain to develop new neuronal interconnections, acquire new functions, and compensate for impairment. These changes are greater if the practice method is meaningful, repetitive, and intensive. It is recommended that rehabilitation take place in stroke care units that can provide an organized package of care through a cyclical process involving assessment, goal setting, intervention, and reassessment. Systematic reviews and meta-analyses have verified the effects of developed techniques in stroke rehabilitation. CIMT that combines a rehabilitative training regime for the paretic limb with constraint of the nonparetic limb can overcome learned nonuse of the paretic limb and has been shown to improve motor function in patients with stroke. BWSTT may induce reorganization at the spinal and supraspinal levels by providing normal gait programs, reducing asymmetries of gait parameters, and increasing walking speed. Robotic training can provide repetitive motor training and reduce the therapists' physical load. Transcutaneous neuromuscular electrical stimulation and NIBS can improve motor recovery by ameliorating use-dependent plasticity impairment after stroke. Moreover, novel stroke rehabilitation strategies such as action observation, VR, and BCI have been developed based on multisensory feedback, which plays an important role in learning to control human brain signals and in re-establishing the sensorimotor loop disrupted by stroke.

Current clinical practice for stroke rehabilitation is based on accumulating evidence from neural plasticity studies. However, responses to rehabilitative interventions show large interindividual variation due to the heterogeneity of mechanisms underlying motor recovery. Therefore, an accurate prediction of motor recovery can help to determine the type, duration, and goals for individual stroke rehabilitation strategies. An assessment of corticospinal integrity using neurophysiological and imaging techniques might be useful for predicting motor recovery and setting individualized rehabilitation goals. However, numerous other factors influence behavioral responses to therapy, including injury to other brain structures, psychosocial factors, and age. Moreover, it is important for appropriate reorganization after stroke to prevent maladaptive plasticity, which weakens motor function and limits motor recovery.

Early stroke rehabilitation is critical for enhancing motor recovery, but the optimal time window for specific neurorehabilitation has yet to be elucidated. The intensity and duration of the rehabilitation strategy are also important factors that influence effectiveness. Although the evidence base for stroke rehabilitation continues to grow, future studies must be conducted to ascertain the optimal time, intensity, and duration for specific rehabilitation techniques and to facilitate the translation of basic scientific evidence into routine clinical application.

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ORIGINAL ARTICLE

Measurement of regional pulse wave velocity using very high frame rate ultrasound

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Abstract
Purpose Pulse wave velocity (PWV) is the propagation velocity of the pressure wave along the artery due to the heartbeat. The PWV becomes faster with progression of arteriosclerosis and, thus, can be used as a diagnostic index of arteriosclerosis. Measurement of PWV is known as a noninvasive approach for diagnosis of arteriosclerosis and is widely used in clinical situations. In the traditional PWV method, the average PWV is calculated between two points, the carotid and femoral arteries, at an interval of several tens of centimeters. However, PWV depends on part of the arterial tree, i.e., PWVs in the distal arteries are faster than those in the proximal arteries. Therefore, measurement of regional PWV is preferable.
Methods To evaluate regional PWV in the present study, the minute vibration velocity of the human carotid arterial wall was measured at intervals of 0.2 mm at 72 points in the arterial longitudinal direction by the *phased-tracking method* at a high temporal resolution of 3472 Hz, and PWV was estimated by applying the Hilbert transform to those waveforms.
Results In the present study, carotid arteries of three healthy subjects were measured in vivo. The PWVs in short segments of 14.4 mm in the arterial longitudinal direction were estimated to be 5.6, 6.4, and 6.7 m/s, which were in good agreement with those reported in the literature. Furthermore, for one of the subjects, a component was clearly found propagating from the periphery to the direction of the heart, i.e., a well known component reflected by the peripheral arteries. By using the proposed method, the propagation speed of the reflection component was also separately estimated to be −8.4 m/s. The higher magnitude of PWV for the reflection component was considered to be the difference in blood pressure at the arrivals of the forward and reflection components.
Conclusion Such a method would be useful for more sensitive evaluation of the change in elasticity due to progression of arteriosclerosis by measuring the regional PWV in a specific artery of interest (not the average PWV including other arteries).

Keywords Regional pulse wave velocity · Parallel beam forming · High frame rate

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Introduction

The number of patients suffering from arteriosclerosis is rapidly increasing with the aging of society and dietary westernization. It is important to make an early diagnosis of arteriosclerosis to prevent serious diseases such as myocardial and cerebral infarction.

Recently, there are many methods for diagnosis of arteriosclerosis, such as angiography, intra-vascular ultrasound (IVUS), optical coherence tomography (OCT), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) [1–3]. These methods are invasive and/or not easily applicable. Therefore, they are unsuitable for repetitive diagnosis to monitor progression of arteriosclerosis. On the other hand, diagnosis with ultrasound is noninvasive, easy to use at bedside, and suitable for repetitive diagnosis.

Ultrasound B-mode imaging is widely used for the morphological diagnosis of the arterial wall, particularly for measurement of intima-media thickness [4–6]. In addition, methods for evaluating the elasticity and viscosity of the arterial wall have recently been developed [7–13]. For diagnosis of early-stage arteriosclerosis, it is useful to evaluate the elastic property of the arterial wall because arterial-wall elasticity changes with progression of arteriosclerosis [14].

For noninvasive clinical evaluation of arterial elasticity, the pulse wave velocity (PWV) method was developed for diagnosis of arteriosclerosis nearly 100 years ago [15, 16]. This method measures the pulse wave, which is the pressure wave generated by the heartbeat, that propagates in the longitudinal direction of the artery. Since PWV c_{PWV} becomes faster with progression of arteriosclerosis, it is used as a diagnostic index of arteriosclerosis and is expressed by the Moens–Korteweg equation as follows [16]:

$$c_{\mathrm{PWV}} = \sqrt{\frac{E_\theta h}{\rho D}}, \tag{1}$$

where E_θ is the Young's modulus in the circumferential direction of the artery, h is the thickness of the arterial wall, ρ is the blood density, and D is the diameter of the artery. The assumptions underlying the Moens–Korteweg equation are that the artery has a thin wall and is filled with an incompressible inviscid liquid. Since PWV is traditionally measured between the carotid artery and femoral artery (cfPWV (carotid-femoral PWV) method) [17, 18] it is not suitable for early diagnosis of arteriosclerosis because lesions of early-stage arteriosclerosis are several millimeters in size. In addition, PWV depends on a part of the arterial tree, i.e., PWV in the distal arteries is faster than that in the proximal arteries. Thus, measurement of regional PWV would be useful for diagnosis of early-stage arteriosclerosis.

We previously studied regional PWVs by measuring small vibration velocities of the arterial wall at two points along the arterial longitudinal direction [19]. To estimate regional PWV more accurately, in the present study, vibration velocities at multiple points in a region of about 14 mm along the arterial longitudinal direction were measured at a high temporal resolution of 3472 Hz. Vibration velocities of the arterial wall have frequency components mostly up to 30 Hz. However, a high temporal resolution is desirable to estimate a small time delay between vibration velocities due to pulse wave propagation, i.e., the pulse wave propagates a region of about 14 mm in 2.8 ms when PWV is 5 m/s.

Materials and methods

Measurement of small vibration velocity for the carotid arterial wall

In the present study, as illustrated in Figs. 1 and 2, minute vibration velocities of the human carotid arterial wall were measured by the *phased-tracking method* [20] at 72 points in the arterial longitudinal direction with intervals of 0.2 mm (total length of 14.4 mm) at a high temporal resolution of 3472 Hz. Modified ultrasonic diagnostic equipment (Aloka, α-10) was used to acquire ultrasonic RF echoes received by each transducer element [21, 22]. The center frequency and fractional bandwidth of an emitted ultrasound pulse were 8 MHz and 61 %, respectively. The received RF echo was sampled at 40 MHz with 16-bit resolution. The method for high frame rate imaging developed by our group [21] was used to obtain beam-formed ultrasonic RF echoes at 72 beam positions at a very high frame rate of 3472 Hz.

Typical PWV is several m/s and passes through a small region of 14.4 mm in a very short period of a few milliseconds. In such a situation, a small random error in estimation of the time delay between velocity waveforms would lead to a significant error in estimation of PWV when PWV is estimated using velocities measured at only two points along the arterial longitudinal direction. Therefore, in the present study, vibration velocities were measured at multiple points to reduce random errors, such as electrical noise and arterial motion estimation error due to random interference of echoes from scatterers in the arterial wall [13].

In the *phased-tracking method*, ultrasonic pulses at angular frequency $\omega_0 = 2\pi f_0$ were transmitted from an ultrasonic transducer placed on the skin surface at a time interval of $\Delta T = 1/f_{\mathrm{FR}}$ (f_{FR}: frame rate) at each ultrasonic beam position. The instantaneous distance between the moving arterial wall and the ultrasonic transducer is denoted by $z(t) = c_0\tau_u(t)/2$, where c_0 is the velocity of ultrasound, and $\tau_u(t)$ is the time delay required for two-way transmission between the ultrasonic transducer and the arterial wall. The phase $\theta(z;t)$ of the quadrature demodulated signal of the received ultrasonic echo is given by $\theta(z;t) = \omega_0\tau_u(t) = 2\omega_0 z(t)/c_0$. The phase difference $\Delta\theta(z;t)$ between time t and $(t + \Delta T)$ is given by

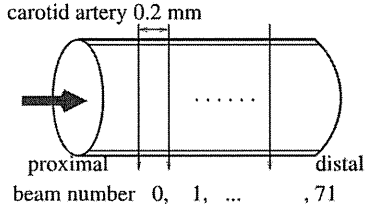

Fig. 1 Illustration of measurement of minute vibration velocity waveforms of the human carotid arterial wall at 72 points at intervals of 0.2 mm

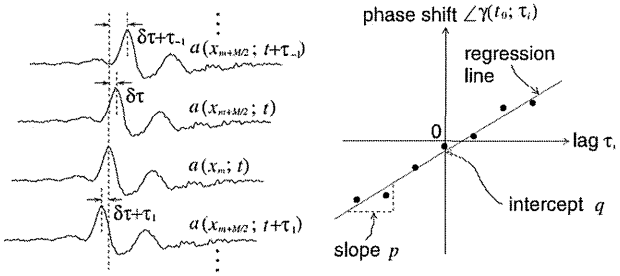

Fig. 2 Illustration of the method for estimation of time delay between acceleration waveforms $a(x_m; t)$ and $\{a(x_{m+M/2}; t + \tau_i)\}$ using their analytic signals

$$\Delta\theta(z;t) = \theta(z;t+\Delta T) - \theta(z;t) = \frac{2\omega_0 \Delta z(t)}{c_0}, \tag{2}$$

where $\Delta z(t) = z(t+\Delta T) - z(t)$ is the displacement of an object during the period ΔT. By dividing the displacement Δz by the period ΔT, the average velocity, denoted by $v(t + \Delta T/2)$, of the object during the period ΔT is given by the phase difference $\Delta\theta(z; t)$ between echoes as follows:

$$v\left(t+\frac{\Delta T}{2}\right) = \frac{\Delta z(t)}{\Delta T} = -\frac{c_0}{2\Delta T}\frac{\Delta\theta(z;t)}{\omega_0}, \tag{3}$$

In the present study, parallel beam forming (PBF) [21] was applied to realize a high frame rate of 3472 Hz. The minute vibration velocity waveforms were measured at 72 points on the human carotid artery wall at intervals of 0.2 mm.

Estimation of PWV by complex correlation approach

In the present study, the acceleration waveforms $\{a(x_m; t)\}$ were obtained by applying time differentiation to the vibration velocity waveforms $\{v(x_m; t)\}$, where x_m is the mth ultrasonic beam position, to enhance high frequency components contained in the measured velocity waveforms because the use of high frequency components improves the time resolution in estimation of time delays among the vibration waveforms due to the propagation of the pulse wave. To estimate PWV, the phase $\phi(x_m; t_0)$ of acceleration waveform $a(x_m; t_0)$ at a time of interest t_0 (=frame) measured at the mth ultrasonic beam position x_m is obtained by applying the Hilbert transform to the acceleration waveforms $\{a(x_m; t)\}$. The Hilbert transform was applied to the acceleration waveforms during just an entire cardiac cycle. The phase $\phi(x_m; t)$ is determined from the complex analytic signal $g(x_m; t)$ of $a(x_m; t)$.

To determine the PWV from the phases of the multiple analytic signals, a method based on the complex cross correlation function was developed in the present study. The complex cross correlation function $\gamma(t_0, \tau_i)$ at t_0 at lag $\tau_i = i \cdot \Delta T$, where $i = -N_l, -N_l + 1, \ldots, -1, 0, 1, \ldots, N_l - 1, N_l$ (N_l denotes the number of lags used in estimation of PWV), is defined as follows:

$$\widehat{\gamma}(t_0, \tau_i) = \sum_{m=0}^{M/2}\sum_{i=-N_c}^{N_c} g^*(x_m; t_0)\cdot g(x_{m+M/2}; t_0 + \tau_i), \tag{4}$$

where $\tau_i = i \cdot \Delta T$, M is the number of ultrasonic beam positions, and N_c determines the number of frames used for calculation of the complex correlation function.

By denoting the original time delay of $g(x_{m+M/2}; t_0)$ from $g(x_m; t_0)$ due to the propagation of the pulse wave in the length of $\delta x \cdot M/2$ (δx: lateral spacing of ultrasonic beams) by $\delta\tau$ and denoting the dominant frequency of $\{g(x_m; t_0)\}$ at t_0 by f_a, $\delta\tau$ is expressed as follows:

$$\delta\tau = -\frac{\angle\gamma(t_0, 0)}{2\pi f_a}. \tag{5}$$

For other lag τ_i, the model $\angle\widehat{\gamma}(t_0, \tau_i)$ of the phase of $\gamma(t_0, \tau_i)$ is expressed as follows:

$$\begin{aligned}\angle\widehat{\gamma}(t_0, \tau_i) &= 2\pi f_a(\tau_i - \delta\tau)\\ &= 2\pi f_a \cdot (i\cdot\Delta T) - 2\pi f_a \delta\tau.\\ &\equiv p\cdot i + q.\end{aligned} \tag{6}$$

To estimate the dominant frequency f_a and time delay $\delta\tau$ due to propagation of the pulse wave, the coefficients p and q are determined by fitting a linear function defined by Eq. (6) to measured $\{\angle\gamma(t_0, \tau_i)\}$ using the least squares method as follows: the difference $\alpha(t_0)$ between the angle of the measured complex correlation function $\angle\gamma(t_0, \tau_i)$ and the model $\angle\widehat{\gamma}(t_0, \tau_i)$ is expressed as follows:

$$\alpha(t_0) = \sum_{i=-N_l}^{N_l} |\angle\gamma(t_0, \tau_i) - (p\cdot i + q)|^2. \tag{7}$$

To determine the coefficients $\widehat{p}$ and $\widehat{q}$, which minimize $\alpha(t_0)$, partially differentiated $\alpha(t_0)$ with respect to p and q are set to zero. By solving the resultant simultaneous equations, the coefficients p and q are determined as follows:

$$\widehat{p} = \frac{(2N_c+1)\left\{\sum_{i=-N_c}^{N_c} i\cdot\angle\gamma(t_0,\tau_i)\right\} - \left(\sum_{i=-N_c}^{N_c} i\right)\left\{\sum_{i=-N_c}^{N_c}\angle\gamma(t_0,\tau_i)\right\}}{(2N_c+1)\left(\sum_{i=-N_c}^{N_c} i^2\right) - \left(\sum_{i=-N_c}^{N_c} i\right)^2}. \tag{8}$$

$$\widehat{q} = \frac{\widehat{p}\sum_{i=-N_c}^{N_c} i - \sum_{i=-N_c}^{N_c}\angle\gamma(t_0,\tau_i)}{(2N_c+1)}. \tag{9}$$

Based on Eq. (6), the dominant frequency f_a is determined using the estimated coefficient $\widehat{p}$ as follows:

$$\widehat{f_a} = \frac{\widehat{p}}{2\pi\Delta T}. \tag{10}$$

Finally, PWV $c_{PWV}(t_0)$ is estimated based on Eq. (6) using the estimated dominant frequency $\widehat{f_a}$ and coefficient $\widehat{q}$ as follows:

$$\begin{aligned}c_{PWV}(t_0) &= \frac{\frac{M}{2}\cdot\delta x}{\widehat{\delta\tau}}\\ &= -\frac{\pi\widehat{f_a}\cdot M\cdot\delta x}{\widehat{q}}.\end{aligned} \tag{11}$$

In Eq. (4), the complex correlation function between analytic signals $\{g(x_m; t_0)\}$ and $\{g(x_{m+M/2}; t_0)\}$ was used because the distance between each pair of the analytic signals should be as large as possible to increase the time delay between the analytic signals and also all the M analytic signals can be used to reduce random errors.

Results

Figure 3a–c shows the B-mode images of 24-(subject A), 23-(subject B), and 38-(subject C) year-old males, respectively. Figures 4, 5, and 6 show in vivo experimental results of subjects A, B, and C, respectively. The short segments of their common carotid arteries of 14.4 mm ((72 beam positions) × (0.2 beam spacing)) were measured. In each of Figs. 4, 5, and 6, electrocardiogram (ECG) (a); the displacement waveform (b), which is obtained by integrating the vibration velocity in (c) with respect to time; vibration velocity measured at the 0th beam position (the 0th beam was nearest to the heart) (c); and the acceleration waveform (d), which is obtained by temporal differentiation of the vibration velocity in (c), are shown. Velocities at the media-adventitia boundaries of the posterior walls were measured as the vibration velocities of the arterial walls.

The vibration of the arterial wall is generated by the ejection of blood from the left ventricle of the heart, and expansion of the artery corresponds to the acceleration waveform around 0.1 s. For example, the enlarged view of the acceleration waveforms of subject A measured at the 0th (proximal) and 71st (distal) beams during a period 0–0.3 s is shown in Fig. 7. As can be seen in Fig. 7, it is recognized that the positive peak around 0.07 s at the 0th beam precedes that of the 71st beam very slightly. In the present study, the large positive peak around $t = 0.1$ s (t_0 for estimation of PWV is shown by the vertical dashed line in Fig. 4), which was caused by ejection of blood from the left ventricle, was analyzed for estimation of PWV.

To estimate PWVs, the Hilbert transform was applied to the acceleration waveforms. The complex cross correlation function defined in Eq. (4) was obtained by setting t_0 to be the time of the peak in the acceleration waveform measured at the 0th beam position around 0.1 s. The assigned t_0 is shown by the vertical dashed lines in Figs. 4, 5, and 6. The length of the correlation window was set at 10 ms (corresponding to $N_c = 17$), which roughly corresponded to the width of the peak in the acceleration waveform. Plots in Fig. 8 show the measured phase $\angle\gamma(t_0, \tau_i)$ of the complex correlation function plotted as a function of the lag τ_i. The PWV was estimated from the slope $\widehat{p}$ and intercept $\widehat{q}$ of the regression line (shown by the dashed line in Fig. 8) based on Eq. (11). By applying the same procedure to the acceleration waveforms of subjects B and C, regional PWVs of subjects A, B, and C were estimated to be 5.6, 6.4, and 6.7 m/s, respectively. The estimated pulse wave velocities were similar to those reported in the literature [23–25].

By observing the acceleration waveform of subject A (shown in Fig. 4d), there is a small but distinct peak around the time of 0.2 s. In the literature, such a peak detected in the acceleration waveform is reported to be a pulse wave component reflected in the distal position of the arterial tree [26–28]. As can be seen in Fig. 7, the acceleration waveform at the 71st beam (nearer to the head) precedes that at the 0th beam very slightly. The PWV for this component was estimated by setting t_0 to the time of the peak in the acceleration waveform measured at the 0th beam position around 0.2 s (t_0 for this reflection component and is shown by the vertical dashed line in Fig. 4). The estimated PWV for the reflection component was −8.4 m/s, where the negative velocity means the propagation from the periphery to the heart. As described above, the regional PWV of the reflection component could also be estimated separately using the proposed method.

Furthermore, for evaluation of the reproducibility of measurements using the proposed method, five additional

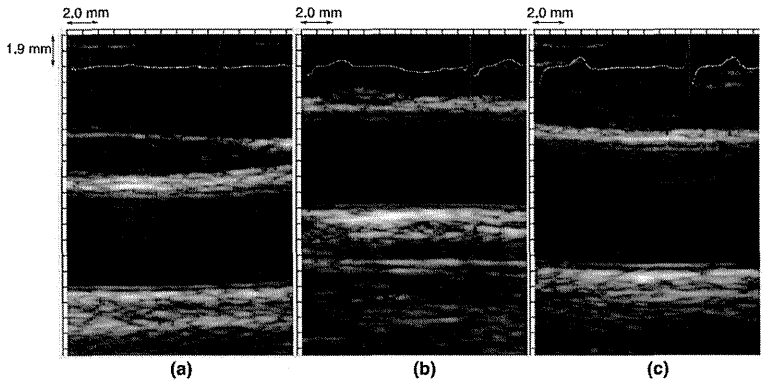

Fig. 3 B-mode images of carotid arteries of 23-(subject A), 24-(subject B), and 38-(subject C) year-old healthy males

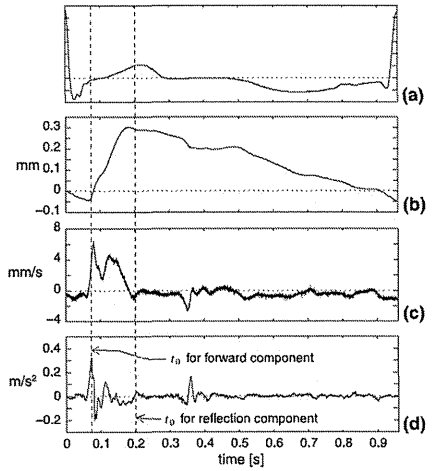

Fig. 4 In vivo experimental results of subject A. **a** Electrocardiogram, **b** displacement, **c** velocity $v(x_m; t)$, and **d** acceleration $a(x_m; t)$ of the posterior wall measured at the 0th ultrasonic beam

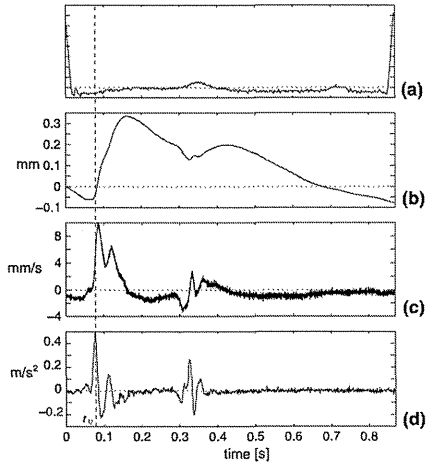

Fig. 5 In vivo experimental results of subject B. **a** Electrocardiogram, **b** displacement, **c** velocity $v(x_m; t)$, and **d** acceleration $a(x_m; t)$ of the posterior wall measured at the 0th ultrasonic beam

measurements were performed with respect to subject C on the same day. Figure 9a shows acceleration waveforms in the 0th scan lines for the five measurements. Using the phase $\angle\gamma(t_0; \tau_i)$ of the complex correlation function shown in Fig. 9b, the regional pulse wave velocities were estimated. The mean and standard deviation for the five measurements were 7.0 and 0.72 m/s (10.4 % of the mean value), respectively.

Discussion

For one of the subjects in the present study, a pulse wave component, which was considered to be reflected by the distal arterial tree, was found. By applying the Hilbert transform and the proposed complex cross correlation technique to the acceleration waveforms obtained by differentiating the measured velocity waveforms, the forward

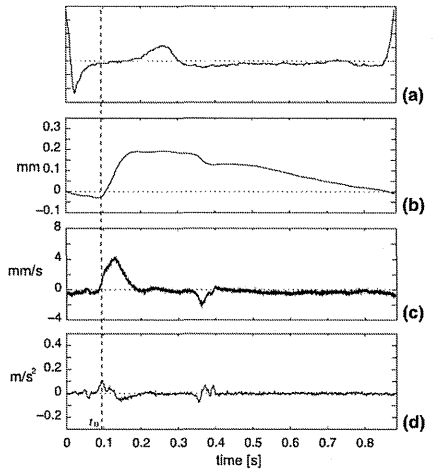

Fig. 6 In vivo experimental results of subject C. **a** Electrocardiogram, **b** displacement, **c** velocity $v(x_m; t)$, and **d** acceleration $a(x_m; t)$ of the posterior wall measured at the 0th ultrasonic beam

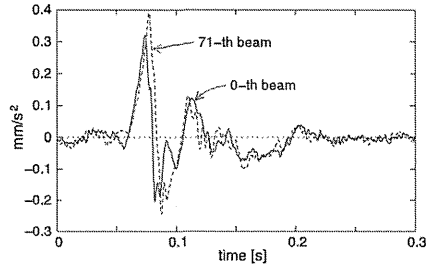

Fig. 7 Enlarged view of the acceleration waveforms $\{a(x_m; t)\}$ of subject A during 0–0.3 s measured at the 0th (*solid line*) and 71th (*dashed line*) ultrasonic beams

and reflection components were separately analyzed. The estimated propagation speed of the forward component was lower (5.6 m/s) than that (8.4 m/s in the opposite direction) of the reflection component. One of the reasons for this difference was considered to be the difference between blood pressures at the arrival times of the forward and reflection components in the carotid artery.

It was reported that the arterial wall distension waveform can be assumed to be the waveform of blood pressure [29–31]. Figure 4b shows the displacement of the posterior arterial wall obtained by integrating the measured velocity

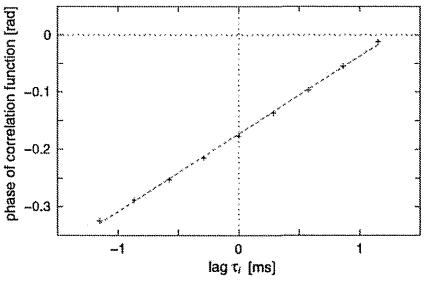

Fig. 8 Phase of complex correlation function $\angle\gamma(t_0, \tau_i)$ plotted as a function of lag τ_i. *Plots* and the *dashed line* show measured phases and the regression line

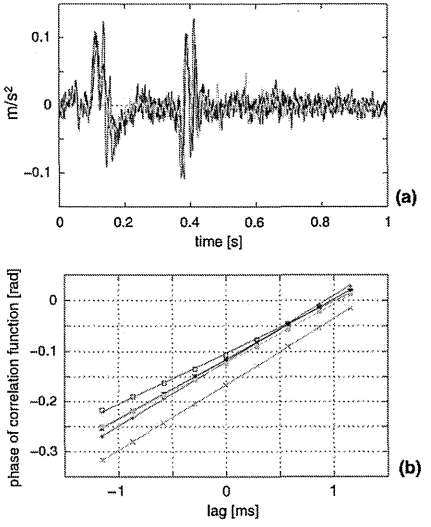

Fig. 9 **a** Acceleration waveforms in the 0th scan lines measured for the five additional measurements with respect to subject C. **b** Phases of complex correlation functions $\{\angle\gamma(t_0, \tau_i)\}$ plotted as a function of lag τ_i. *Plots* and *lines* show measured phases and the regression lines

waveform with respect to time. At the time (around 0.1 s) of the peak of the forward component in the acceleration waveform shown in Fig. 4d, the magnitude of the displacement is nearly zero. This fact suggests that blood pressure at the arrival of the forward component corresponds to the diastolic blood pressure. On the other hand, the displacement at the time of the arrival of the reflection component is nearly maximum, which suggests that blood

pressure at the time of arrival of the reflection component is nearly the systolic blood pressure. It is also reported that the stress–strain relationship of the arterial wall is nonlinear [32–35], and the elastic modulus of the arterial wall at higher blood pressure is larger than that at lower blood pressure. Therefore, the PWV of the reflection component (presumably at higher blood pressure) was considered to be larger than that at the forward component.

In the present study, the propagation of pulse waves in a local region in the carotid artery was analyzed in detail by measuring vibration velocities of the arterial wall at a very high temporal resolution of 3472 Hz. To measure the regional PWV in an arterial longitudinal segment of about 15 mm, a small time delay of a few milliseconds between vibration waveforms of the arterial wall needs to be estimated. For this purpose, in the present study, acceleration waveforms, which contain higher frequency components compared with displacement waveforms, were measured at a high temporal resolution, and a method for estimation of a small time delay using the phase of the acceleration waveform was developed.

Figures 10b–d show the displacement, velocity, and acceleration waveforms sampled at a frame rate of 35 Hz (waveforms in Fig. 4 were down sampled), which is a typical frame rate of conventional ultrasonic diagnostic equipment. As shown in Fig. 10, it is difficult to measure the velocity and acceleration waveforms at a temporal resolution of 35 Hz. Using the displacement waveform shown in Fig. 10d, which was sampled at 35 Hz, the pulse wave velocity was also estimated by the proposed method described in "Estimation of PWV by a complex correlation approach". The estimated PWV was −37.2 m/s, which was significantly different from that reported in the literature. Therefore, it is necessary to measure the vibration waveform at a high temporal resolution.

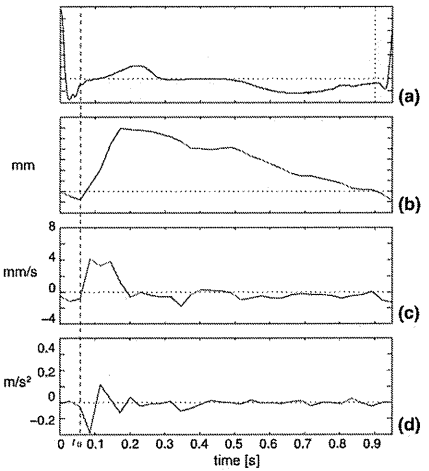

Fig. 10 Vibration waveforms of subject A (waveforms in Fig. 4 are down sampled at 35 Hz). **a** Electrocardiogram, **b** displacement, **c** velocity $v(x_m; t)$, and **d** acceleration $a(x_m; t)$ of the posterior wall measured at the 0th ultrasonic beam

As described above, in the present study, regional pulse wave velocities were noninvasively measured in vivo by the proposed method and compared with those reported in the literature. In future studies, it will be necessary to evaluate the accuracy of estimation of regional PWV using the proposed method by basic experiments using phantoms. In addition, the conditions of the spacing and number of scan lines and frame rate, etc., required for measurement of regional PWV also need to be investigated by phantom experiments. However, the results obtained in the present study show the possibility of the proposed method for measurement of regional PWV, and such a method would be useful for diagnosis of arteriosclerosis.

Conclusion

For detailed analyses of pulse wave propagation in a local region of 14.4 mm in the carotid artery, in the present study, small vibrations on the arterial wall were measured with ultrasound at a very high frame rate of 3472 Hz. By applying the Hilbert transform to the acceleration waveforms of the arterial wall, which were obtained by differentiating the measured vibration velocity waveforms, pulse wave velocities of three healthy subjects were estimated using the proposed complex cross correlation approach. Furthermore, in one of the subjects, there was a component propagating from the heart to the periphery and from the periphery to the heart. Even in such a case, pulse wave velocities for these forward and reflection components were separately estimated, and the magnitudes of the estimated pulse wave velocities were similar to those reported in the literature. Such a method for measurement of regional PWV would be useful for more sensitive evaluation of the change in elasticity due to progression of arteriosclerosis.

Conflict of interest None.

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Inverse association between circulating adiponectin levels and skeletal muscle strength in Japanese men and women

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KEYWORDS
Adiponectin;
Grip strength;
Leg extension power;
Adults

Abstract *Background and aims:* Increased levels of circulating adiponectin in the elderly cause a negative impact on physical function and health status, which suggests that circulating adiponectin may be related to skeletal muscle function. However, data on the relationship between circulating adiponectin levels and skeletal muscle function is limited. Our objective was to investigate the association between serum adiponectin levels and muscle strength in adults. *Methods and results:* This cross-sectional study is a part of the Oroshisho Study of adult employees in Japan from 2008 to 2011. In our study, we used data gathered in 2008–2010 that had included serum adiponectin measurements (n = 1378; age, 19–83 years). From this population, 1259 subjects were evaluated for grip strength (949 men, 310 women), and 965 subjects were evaluated for leg extension power (716 men, 249 women). Multivariate linear regression analyses showed that adiponectin was associated significantly and negatively with both grip strength (β and standard error [SE]: men, −0.09 [0.01], p = 0.010; women, −0.20 [0.03], kg, p = 0.002) and leg extension power (men, −0.09 [0.02], p = 0.014; women, −0.14 [0.07], W, p = 0.032) after adjusting for age, physical activity, nutrient intake, depressive symptoms, metabolic syndrome, C-reactive protein, body mass index, and other lifestyle-related potential confounders.

Abbreviations: ANCOVA, analysis of covariance; BMI, body mass index; CVD, cardiovascular disease; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high-sensitivity C-reactive protein; MET, metabolic equivalent; PA, physical activity; SE, standard error.

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Conclusion: This population-based cross-sectional study indicates an inverse association between serum adiponectin levels and muscle strength in adults. Further studies are necessary to confirm this association and to clarify causality.

Introduction

Adiponectin, an adipocyte hormone produced mainly by the adipose tissue that regulates energy homeostasis, circulates abundantly in the blood [1]. Adiponectin differs from other adipokines because it is reduced in obesity and increases energy expenditure [1]; thus, adiponectin may induce weight loss by acting on the brain directly [2]. In addition, a human-based nested case–control study indicated that high levels of circulating adiponectin in adult men are associated with a lower risk for myocardial infarction, which suggests that adiponectin may be protective against coronary heart disease [3].

However, there is a growing interest in the possibility that high levels of adiponectin may play a paradoxical role in the pathogenesis of cardiovascular disease (CVD) and/or coronary heart disease and CVD-related mortality. Several human-based epidemiologic studies confirmed this hypothesis by demonstrating that high adiponectin levels increase the risk of CVD and mortality among elderly [4–6] and predominantly middle-aged populations [7]. Although the precise mechanisms underlying this relationship are unknown so far, Wannamethee et al. [4,8] hypothesized that adiponectin may accelerate the decline of skeletal muscle function, which has been linked to an increased risk of mortality. This hypothesis was based on two human-based prospective studies, which demonstrated an inverse relationship of circulating adiponectin level with physical function, including muscle function [9,10]. Moreover, muscle strength is an important indicator of muscle function and is related inversely to CVD incidence in adults [11]; further, it is a significant predictor of CVD risk and mortality due to all causes in the elderly [11].

Recently, a significant and independent association between high adiponectin levels and physical disability in the elderly has been observed [9]; Hozawa et al. [10] also reported a positive association between circulating adiponectin and physical disability in 505 Japanese individuals aged 70 years or more. These findings suggest potential involvement between circulating adiponectin and skeletal muscle function; however, little is known about this relationship.

Therefore, in the current study, a cross-sectional study in Japanese adults was designed to investigate the association between sex-specific serum adiponectin levels and skeletal muscle strength, including grip strength and leg extension power.

Methods

Study population

A cross-sectional study was performed using data gathered in 2008–2010 from the Oroshisho Study, a longitudinal study of the lifestyle-related effects on illness and health status in Japanese adult employees working at the Sendai Oroshisho Center in Sendai, Japan, between 2008 and 2011. Details of the Oroshisho Study have been described elsewhere [12].

Current analyses were limited to participants who provided written informed consent for the analysis of their data (n = 1619). Of this group, 3 were excluded because they lacked serum adiponectin data, 194 were excluded because they had a history of CVD, renal failure, or the use of anti-hypertensive, lipid-lowering, or anti-diabetic agents, and 44 were excluded due to incomplete data, including information on their physical activity, dietary habits, depressive symptoms, occupation, and education levels. Based on this sample of participants, subjects with data missing for grip strength (n = 119) or leg extension power (n = 413) were also excluded from these analyses. For grip strength analysis, a total of 1259 individuals (949 men and 310 women) had provided data for all the variables studied. For leg extension power analysis, 965 subjects (716 men and 249 women) were available. All research procedures in the current study were consistent with ethical principles for medical research involving human subjects from the Declaration of Helsinki [13]. The Institutional Review Board of the Tohoku University Graduate School of Medicine approved the protocol for the study.

Adiponectin and other biochemical analyses

Blood samples were drawn simultaneously from the antecubital veins of patients at rest before breakfast and collected in siliconized vacuum glass tubes until analysis. Serum adiponectin levels were determined by a specific sandwich enzyme-linked immunosorbent assay (Otsuka Pharmaceutical, Tokyo, Japan); the lower limit of detection was 23.4 pg/mL. The detection range of adiponectin was 0.375–12.0 ng/mL, and its intra- and inter-assay coefficients of variation were <10%.

Serum high-sensitivity C-reactive protein (hs-CRP) levels were measured by N-latex CRP-2 (Siemens Healthcare Japan, Tokyo, Japan). Enzymatic methods were used via kits to measure fasting blood glucose (Eurotec, Tokyo, Japan), triglycerides, and high-density lipoprotein-cholesterol levels (Sekisui Medical, Tokyo, Japan).

Assessment of skeletal muscle strength

Grip strength (measured in kg) using an adjustable Smedley dynamometer (TKK 5401; Takei Scientific Instruments Co., Ltd., Niigata, Japan) on subjects who were maintained in a standing position. The dynamometer was adjusted individually to fit the hand size, and the subjects were encouraged to exert maximal grip effort. Two measurements were

recorded for each hand with a brief interval between trials. The mean of the highest value for each hand was used for analysis.

Maximal bilateral leg extension power (W) was determined by using an isotonic apparatus (Anaeropress 3500; Combi Co., Tokyo, Japan). After warming up, the subjects reclined on the seat and placed both feet on the footplate at a knee angle of 90°. The load of the footplate was set to the subject's weight. Subjects pressed their feet horizontally as intensely as possible until their legs extended fully. Five trials were performed at 15-s intervals, and the highest value was chosen for inclusion in the analyses. The reliability and validity of the leg extension power measurement were evaluated and described in details elsewhere [14].

Relevant covariates

Height, weight, and waist circumference were measured according to a standardized protocol. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Blood pressure was measured twice consecutively from the upper right arm by an automatic device (Yamasu 605P; Kenzmedico, Saitama, Japan) after a 5 min period of rest in a seated position. The mean of the 2 measurements was used as the blood pressure value. The International Physical Activity Questionnaire was used to assess and calculate total physical activity (PA) [15]. PA was divided into 2 categories, <23 and ≥23 metabolic equivalent (MET) h/week [16]. More than 23 MET h/week is the reference quantity of exercise, physical activity, and fitness level for prevention of lifestyle-related diseases, which recommended by the Ministry of Health, Labour and Welfare of Japan [16]. Daily dietary energy and protein intake was estimated for the past month based on the results of a brief self-administered diet history questionnaire [17]. The Japanese version of the Self-Rating Depression Scale was used to examine the severity of depression subjectively [18]. Participants who scored ≥45 raw sum points were considered to be suffering from depression [19]. Metabolic syndrome was defined according to recent guidelines from the American Heart Association Scientific Statements of 2009 for people of Asian ethnicity (including Japanese) [20]. In addition, age, sex, smoking status (never, former, or current), drinking frequency (never, 1–6 drink(s)/week, or 7 drinks/week), education levels (<college or ≥college), and occupation (desk-centric or not) were gathered via a self-administered questionnaire survey.

Statistical analysis

The distributions of all continuous variables in this study were skewed positively; therefore, we normalized by log-transforming the data in our analyses. Data were expressed as means (standard deviation) or geometric means (95% confidence interval) for continuous variables and as percentages for categorical variables. Participant characteristics with regard to circulating adiponectin levels were assessed by using linear regression models adjusted for age. Associations between adiponectin and muscle strength were determined by simple correlation using Pearson's correlation coefficient (r). For multivariate analyses, linear regression models or analysis of covariance (ANCOVA) were used, and the initial model was adjusted for age, occupation, and education levels (Model 1); Model 2 was additionally adjusted for smoking status, drinking frequency, PA, depressive symptoms, metabolic syndrome, energy intakes, protein intakes, and hs-CRP, whereas, Model 3 was additionally adjusted for BMI. All variables that were at a level of significance of $p < 0.10$ in univariate analyses were included in multivariate models. Additionally, variables which were significantly associated with muscle strength, such as age, occupation, education levels, and daily energy intake were considered as potential confounding factors (data not shown). Furthermore, depressive symptoms [21] and daily protein intake [12] were included in the multivariate models because of their close association with adiponectin levels in the previous studies. For multivariate linear regression analyses, the values were expressed as β and standard error (SE). For ANCOVA, adiponectin levels were categorized into quartiles based on the distribution of adiponectin concentrations, and geometric mean values were used to test for linear trends in increasing quartiles. A secondary analysis was performed to evaluate the associations between serum adiponectin and muscle strength relative to body weight [22]. All statistical tests were two-tailed; p values less than 0.05 were considered statistically significant. Data were analyzed with IBM SPSS Statistics 19.0 software (IBM SPSS Inc., Chicago, IL, USA).

Results

Table 1 displays age-adjusted population characteristics based on the levels of circulating adiponectin in men. As expected, the serum adiponectin levels in both the muscle strength groups correlated negatively with weight, BMI, metabolic syndrome, and levels of hs-CRP, triglycerides, and fasting glucose; they correlated positively with high-density lipoprotein-cholesterol levels. Additionally, significantly higher levels of adiponectin were detected in men who reported either PA ≥23 MET h/week (a low level of PA was used as the reference) or daily drinking (no drinking was used as the reference). Lower levels of adiponectin were detected in current smokers (no smoking was used as the reference). However, no significant associations were detected between adiponectin levels and age, desk-centric work, education levels, depression symptoms, former smoking status, occasional drinking, and daily energy and protein intakes. In the analyses on women, no associations emerged between adiponectin levels and smoking status (Table 2). Otherwise, the results for women were similar to those for men.

To determine the association between adiponectin levels and muscle strength, several analyses were performed. The univariate Pearson's correlation coefficients (Table 3) showed that serum adiponectin levels were associated significantly and negatively with absolute muscle strength in both sexes ($p < 0.001$), whereas such associations disappeared or inverted when muscle strength divided by body weight. In the multivariate linear regression analyses (Table 4), a statistically significant, negative association of adiponectin with muscle strength after adjusting for potential confounding factors was found

Table 1 Age-adjusted participant characteristics according to circulating adiponectin levels in men.[a]

Variables	Grip strength group (n = 949)				Leg extension power group (n = 716)			
	Value[b]	β (SE)	R^2	p	Value	β (SE)	R^2	p
Adiponectin, mg/L[c]	6.7 (3.2)				6.8 (3.4)			
Grip strength, kg	43.8 (6.5)							
Grip strength, kg/kg	0.62 (0.10)							
Leg extension power, W					1264 (371)			
Leg extension power, W/kg					18.6 (4.9)			
Age, years	44.2 (10.3)	0.02 (0.07)	0.001	0.470	44.3 (10.5)	0.05 (0.08)	0.001	0.154
Weight, kg	68.7 (10.9)	−0.32 (0.10)	0.098	<0.001	68.5 (11.2)	−0.30 (0.12)	0.086	<0.001
BMI, kg/m^2	23.7 (3.4)	−0.36 (0.11)	0.129	<0.001	23.6 (3.5)	−0.34 (0.13)	0.114	<0.001
Desk work, %	57.9	0.01 (0.03)	0.001	0.735	53.4	−0.02 (0.04)	0.000	0.652
Education (≥college), %	36.8	−0.02 (0.03)	0.001	0.467	37.4	−0.02 (0.04)	0.000	0.618
Metabolic syndrome, %	18.8	−0.27 (0.04)	0.073	<0.001	17.9	−0.26 (0.05)	0.066	<0.001
Depressive symptoms, %	33.6	0.02 (0.03)	0.001	0.521	33.9	0.03 (0.04)	0.000	0.445
Smoking status								
Never, %	33.2	Reference			35.5	Reference		
Former, %	12.3	0.00 (0.05)	0.000	0.992	13.3	−0.01 (0.06)	0.001	0.755
Current, %	54.5	−0.10 (0.04)	0.009	0.003	51.3	−0.11 (0.04)	0.011	0.006
Drinking frequency								
Never, %	17	Reference			17.9	Reference		
1–6 drink(s)/week, %	52.1	0.03 (0.04)	0.000	0.574	51.3	0.10 (0.05)	0.000	0.052
7 drinks/week, %	31	0.09 (0.05)	0.004	0.040	30.9	0.14 (0.06)	0.005	0.006
PA, MET h/week								
<23, %	62.4	Reference			62.7	Reference		
≥23, %	37.6	0.07 (0.03)	0.003	0.039	37.3	0.07 (0.04)	0.005	0.072
Daily energy intake, kcal	1955 (604)	−0.03 (0.05)	0.000	0.315	1988 (614)	−0.06 (0.06)	0.003	0.132
Daily protein intake, g	65.7 (24.7)	−0.02 (0.04)	0.001	0.582	66.8 (25.1)	−0.03 (0.05)	0.001	0.404
High-sensitivity CRP, mg/L	0.98 (3.54)	−0.32 (0.01)	0.101	<0.001	0.83 (2.37)	−0.35 (0.02)	0.119	<0.001
Triglyceride, mg/dL	138 (105)	−0.39 (0.03)	0.144	<0.001	132 (94)	−0.38 (0.03)	0.144	<0.001
HDL-C, mg/dL	53.0 (12.8)	0.38 (0.06)	0.146	<0.001	53.7 (12.9)	0.42 (0.07)	0.178	<0.001
Fasting glucose, mg/dL	95.1 (17.4)	−0.11 (0.12)	0.009	0.001	94.4 (13.9)	−0.13 (0.16)	0.016	0.001

Abbreviations: BMI, body mass index; PA, physical activity; CRP, C-reactive protein; HDL-C, high-density lipoprotein-cholesterol.
[a] Analyzed by multiple regression analysis with all values adjusted for age.
[b] Data are summarized by mean (standard deviation) for continuous variables and by percentage for category variables.
[c] All continuous variables have been log-transformed.

($p < 0.05$ for all). There were no determinant changes in R^2 between each multivariate model except in grip strength/body weight (kg/kg) group (Model 1 to Model 2: men, 0.135; women, 0.182; Model 2 to Model 3: men, 0.216; women, 0.210) (data not shown). In ANCOVA analyses, all of these associations were similar in men and women (Fig. 1).

Discussion

A cross-sectional study was conducted in Japanese adult men and women to assess the association between circulating adiponectin levels and skeletal muscle strength. Multivariate analyses showed high adiponectin levels to be significantly associated with lower muscle strength in both sexes.

These findings agree with 2 recent prospective studies that showed a significant association between high adiponectin levels and the decline of physical function in elderly subjects [9,10]. The first study [9] was a human-based, long-term, longitudinal cohort that included 988 elderly persons and indicated a significant positive association between adiponectin levels and physical disability even with the use of the adjusted model that included prior weight loss. Similar findings emerged subsequently in a 6 years follow-up study from the Tsurugaya Project on 505 Japanese individuals aged 70 years or more [10]. Furthermore, our findings are consistent with the results from the most recent AFINOS study [23], a small cross-sectional study reporting that serum adiponectin levels were associated significantly and inversely with muscular fitness after adjusting for age, sex, pubertal status, and waist circumference in 198 adolescents aged 13–17 years. Compared to the AFINOS study, our study extends the link between circulating adiponectin levels and muscle strength to include the general adult population aged 19–83 years. These findings imply that the negative association between adiponectin levels and muscle function is unrelated to age.

Whether adiponectin directly affects skeletal muscle function is unknown; however, several possible mechanisms have been explored here to explain the observed inverse association between serum adiponectin levels and muscle strength. First, one of possible explanations for this relationship is found in a report by Ingelsson et al. [24], which

Table 2 Age-adjusted participant characteristics according to circulating adiponectin levels in women.[a]

Variables	Grip strength group (n = 310)				Leg extension power group (n = 249)			
	Value[b]	β (SE)	R^2	p	Value	β (SE)	R^2	p
Adiponectin, mg/L[c]	10.7 (4.4)				11.0 (4.5)			
Grip strength, kg	25.4 (4.5)							
Grip strength, kg/kg	0.49 (0.09)							
Leg extension power, W					502 (208)			
Leg extension power, W/kg					9.5 (3.7)			
Age, years	42.2 (10.2)	−0.05 (0.10)	0.002	0.390	41.7 (10.4)	0.00 (0.11)	0.000	0.998
Weight, kg	53.5 (10.4)	−0.35 (0.13)	0.116	<0.001	52.9 (9.8)	−0.31 (0.15)	0.085	<0.001
BMI, kg/m^2	21.5 (3.8)	−0.38 (0.14)	0.136	<0.001	21.2 (3.5)	−0.32 (0.17)	0.092	<0.001
Desk work, %	84.2	0.00 (0.07)	0.002	0.942	86.3	−0.01 (0.08)	0.000	0.822
Education (≥college), %	11.6	0.07 (0.07)	0.001	0.218	12.9	0.06 (0.08)	0.003	0.362
Metabolic syndrome, %	9.7	−0.33 (0.08)	0.102	<0.001	8.0	−0.23 (0.10)	0.043	<0.001
Depressive symptoms, %	39.7	−0.03 (0.05)	0.004	0.548	41.4	−0.10 (0.05)	0.005	0.105
Smoking status								
Never, %	69.7	Reference			66.7	Reference		
Former, %	8.7	0.05 (0.09)	0.000	0.378	9.2	0.04 (0.09)	0.002	0.551
Current, %	21.6	−0.08 (0.06)	0.003	0.180	24.1	−0.04 (0.06)	0.003	0.499
Drinking frequency								
Never, %	41.0	Reference			39.4	Reference		
1–6 drink(s)/week, %	50.0	0.00 (0.05)	0.002	0.972	51.0	0.01 (0.06)	0.002	0.926
7 drinks/week, %	9.0	0.14 (0.09)	0.015	0.021	9.6	0.14 (0.10)	0.011	0.036
PA, MET h/week								
<23, %	69.7	Reference			71.5	Reference		
≥23, %	30.3	0.16 (0.05)	0.020	0.006	28.5	0.16 (0.06)	0.017	0.012
Daily energy intake, kcal	1594 (599)	0.03 (0.07)	0.003	0.641	1602 (617)	0.04 (0.08)	0.001	0.584
Daily protein intake, g	56.5 (24.5)	0.03 (0.06)	0.004	0.550	57.1 (25.5)	0.01 (0.06)	0.000	0.826
High-sensitivity CRP, mg/L	0.70 (2.65)	−0.34 (0.02)	0.108	<0.001	0.60 (2.67)	−0.30 (0.02)	0.079	<0.001
Triglyceride, mg/dL	73.0 (49.8)	−0.29 (0.05)	0.070	<0.001	71.4 (50.9)	−0.30 (0.06)	0.069	<0.001
HDL-C, mg/dL	63.6 (13.2)	0.48 (0.10)	0.229	<0.001	64.3 (13.3)	0.48 (0.11)	0.221	<0.001
Fasting glucose, mg/dL	88.5 (10.8)	−0.22 (0.23)	0.037	<0.001	87.7 (9.1)	−0.16 (0.28)	0.014	0.020

Abbreviations: BMI, body mass index; PA, physical activity; CRP, C-reactive protein; HDL-C, high-density lipoprotein-cholesterol.
[a] Analyzed by multiple regression analysis with all values adjusted for age.
[b] Data are summarized by mean (standard deviation) for continuous variables and by percentage for category variables.
[c] All continuous variables have been log-transformed.

demonstrates that the proportion of type IIb muscle fibers decreases with higher adiponectin levels in elderly men after adjusting for several potential confounders. In a most recent study, a negative association of circulating adiponectin level with muscle fiber size independent of age and total adiposity among healthy middle-aged men was also indicated [25]. It is well known that type II muscle fiber size [26] and muscle hypertrophy [27] are considered as the main predictors of muscle strength. Therefore, to some extent, muscle fiber size and morphology have become important to explain the results observed in this study; further research is required on this topic. Furthermore, inflammatory responses may also play a role in this significant inverse association between serum adiponectin and muscle strength, although the results were not changed after adjustment for hs-CRP. It is reported that adiponectin levels were inversely associated with inflammatory marker CRP concentrations in substantially healthy Japanese men [28]. Schaap et al. [29] showed higher CRP in older persons was associated with declines in muscle mass and strength. Apart from these, it is worth to note that negative association was also found between adiponectin levels and muscle strength relative to body weight in multivariate model while significant results disappeared or inverted in univariate analysis, which suggests the involvement of body weight in this association. On the other hand, adjustment for BMI in our study did not change the negative association

Table 3 Pearson's correlation coefficient (r) between circulating adiponectin levels and muscle strength.[a]

	Men	Women
Grip strength, kg	−0.124*	−0.165†
Grip strength/body weight, kg/kg	0.165*	0.145‡
Leg extension power, W	−0.174*	−0.181†
Leg extension power/body weight, W/kg	−0.021	−0.071

Notes: The sample for grip strength analyses included 949 men and 310 women; for leg extension power analyses included 716 men and 249 women. *p value < 0.001; †p value < 0.01; ‡p value < 0.05.
[a] All variables have been log-transformed.

Table 4 Multivariate linear regression analysis on the association between circulating adiponectin levels and muscle strength.[a]

	Model 1			Model 2			Model 3		
	β (SE)	R^2	p	β (SE)	R^2	p	β (SE)	R^2	p
Grip strength, kg									
Men (n = 949)	−0.12 (0.01)	0.051	<0.001	−0.13 (0.01)	0.081	<0.001	−0.09 (0.01)	0.115	0.010
Women (n = 310)	−0.18 (0.02)	0.035	0.002	−0.23 (0.03)	0.088	<0.001	−0.20 (0.03)	0.114	0.002
Grip strength/body weight, kg/kg									
Men (n = 949)	0.16 (0.01)	0.032	<0.001	0.02 (0.01)	0.171	0.505	−0.09 (0.01)	0.387	0.003
Women (n = 310)	0.14 (0.03)	0.017	0.016	−0.07 (0.03)	0.212	0.214	−0.17 (0.03)	0.422	0.001
Leg extension power, W									
Men (n = 716)	−0.16 (0.02)	0.179	<0.001	−0.14 (0.02)	0.217	<0.001	−0.09 (0.02)	0.285	0.014
Women (n = 249)	−0.19 (0.07)	0.045	0.003	−0.19 (0.07)	0.136	0.005	−0.14 (0.07)	0.194	0.032
Leg extension power/body weight, W/kg									
Men (n = 716)	−0.01 (0.02)	0.098	0.857	−0.08 (0.02)	0.154	0.044	−0.10 (0.02)	0.171	0.007
Women (n = 249)	−0.08 (0.06)	0.014	0.242	−0.13 (0.07)	0.106	0.048	−0.14 (0.07)	0.107	0.041

Notes: Model 1, adjusted for age, occupation, and education levels; Model 2, same as Model 1 plus smoking status, drinking frequency, physical activity, energy intakes, protein intakes, depressive symptoms, metabolic syndrome, and high-sensitivity C-reactive protein; Model 3, same as Model 2 plus body mass index.
[a] All continuous variables have been log-transformed.

between adiponectin levels and either grip strength or leg extension power, which suggests that BMI did not account, at least as a principal mechanism, for the results.

In current study, among a large number of covariates in the multivariate analyses, daily protein intakes were considered as an important one because an earlier report from the Oroshisho Study [12] indicated that the protein centric "Izakaya" dietary pattern, which characterized by a high consumption of meat, fish and alcohol, was associated with lower serum adiponectin levels. Moreover, in a prospective study, high-meat dietary pattern in the elderly tended to reduce the risk of fall-related fracture, whereas a positive association between vegetable pattern and the fall-related fracture risk was observed [30]. These data suggested that protein intakes play a role in both circulating adiponectin concentrations and physical function, although it failed to explain the inverse association between serum adiponectin levels and muscle strength in the present study.

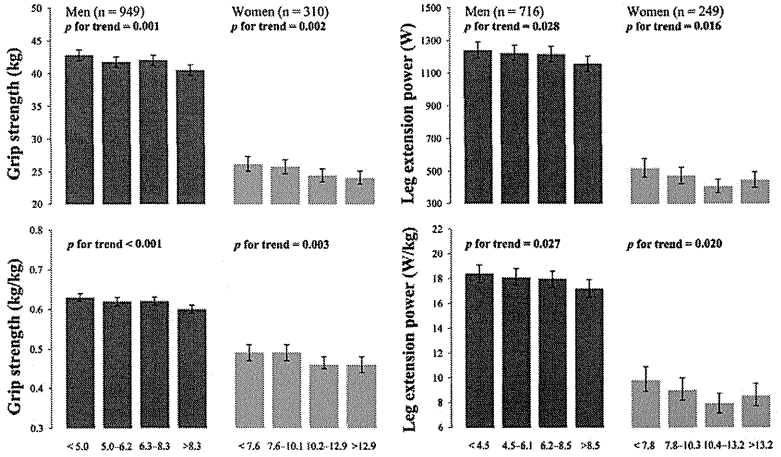

Figure 1 Multivariable-adjusted geometric means and 95% confidence intervals of grip strength and leg extension power according to serum adiponectin quartiles (mg/L). Confounding factors include age, occupation, education levels, smoking status, drinking frequency, physical activity, energy and protein intakes, depressive symptoms, metabolic syndrome, high-sensitivity C-reactive protein levels, and body mass index.