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Electrical Stimulation of the Abdomen Preserves Motor Performance in the Inactive Elderly: A Randomized Controlled Trial

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Abdominal muscle strength declines easily with the process of aging and/or disuse, and it is difficult to strengthen weak abdominal muscles in the inactive elderly. In the present study, we applied surface electrical stimulation (ES) to the abdomen of inactive elderly people to investigate its chronic effects. Twenty inactive elderly people (65-89 years) who spent most of the day in their bedroom participated in the study. The subjects were assigned to ES and non-ES groups in a random order. In addition to conventional physical therapy and occupational therapy, ES was applied to both sides of the flank of 10 subjects (ES group) for 8 weeks. For evaluation of the abdominal muscles, the cross-sectional area (CSA) was measured with computed tomography and the electrical muscle activity (iEMG) was measured by electromyography. Functional examinations were performed at 2, 4, and 8 weeks after the beginning of the study with the following parameters: grip strength; maximum walking speed (WS); movement time for sitting up (MSU); number of trunk flexions (NTF); flexibility of the trunk; sit-to-stand time (STS); and Barthel index (BI) score. In the ES group, the NTF and MSU were significantly improved at 4 weeks and thereafter. Furthermore, the STS and WS were also improved significantly after 8 weeks ($p < 0.05$). The CSA and iEMG both increased significantly ($p < 0.05$). However, the flexibility of the trunk and BI score did not change. In conclusion, ES to the abdomen has the potential to improve motor function in the inactive elderly.

Keywords: abdominal muscles; inactive elderly; motor function; rehabilitation programs; surface electrical stimulation
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In recent years, with the increasing number of elderly people in Japan, preventive care has become of considerable importance (Ishibashi and Ikagami 2010). Loss of muscle strength with aging has been suggested to be one of the main factors causing functional limitations in activities of daily living (ADL), such as rising from a chair or climbing up and down stairs (Guralnik et al. 1995; Odding et al. 1995; Schenkman et al. 1996). The strength of the abdominal muscles declines easily with the process of aging and/or disuse (Chen and Kuo 1989; Buchman et al. 2009), and these muscles have important roles for not only trunk function, but also the whole body motor function (Buchman et al. 2008; Simões et al. 2010). The subscore of the functional independence measures relating to locomotion transfers in stroke subjects at discharge was reported to be positively correlated with the trunk muscle torque values, except for isometric extension (Karatas et al. 2004). However, it is difficult to strengthen weak abdominal muscles in the inactive elderly.

Machine training, aerobics, and other activities are recommended for health promotion in the elderly, but there are few programs available for inactive elderly people to strengthen their weak abdominal muscles (Freiberger et al. 2011). Some methods of physical therapy, including electrical stimulation (ES) (DiMarco and Kowalski 2008), have been used for muscle strengthening (Gerovasili et al. 2009). In particular, ES has been adopted to improve not only muscle atrophy, but also impaired motor function in hemiplegic patients (Gerovasili et al. 2009) and spinal cord-injured patients (Popovic et al. 2006; Popović et al. 2009) since the 1980s, and its safety and effectiveness have been confirmed (Gordon and Mao 1994; Hillegass and Dudley 1999; Winslow et al. 2003; Coghlan et al. 2008; Ochi et al. 2010). Furthermore, it was reported that ES of the abdo-

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Table 1. Demographic and clinical parameters of the subjects.

	ES group (n = 10)	Ctrl group (n = 10)	p
Age	78.5 ± 13.5	73.5 ± 8.2	.16
Height (cm)	152.2 ± 9.8	154.8 ± 12.1	.83
Weight (kg)	55.1 ± 10.4	49.9 ± 10.3	.17
BMI (kg/m^2)	23.7 ± 2.9	20.9 ± 3.7	.17
BI	53.8 ± 20.8	56.9 ± 21.4	.67
Time from onset to start of the study (months)	26.3 ± 13.5	27.3 ± 21.0	.75

Values are presented as the mean ± S.D.
No significant differences between the two groups were noted for the characteristics at baseline.
BMI, body mass index; BI, Barthel index.

men improved the function of the abdominal muscles in tetraplegic patients (DiMarco et al. 2008; Lee et al. 2008). In the present study, we applied surface ES to the abdominal muscles of inactive elderly people and investigated its local and general effects, such as changes in the abdominal muscle activity and motor performance. We also investigated the changes in the volume of the abdominal muscles after ES.

Methods

Subjects and overall design

This prospective randomized study was carried out in 20 elderly people (9 males and 11 females) who were admitted to an institute or a hospital for nursing care. We defined inactive elderly people as those who spent most of the day in their bedroom (Nashimoto et al. 2002). The subjects had prolonged decreases in muscle contractile activity, such as those associated with a sedentary lifestyle, periods of prolonged bed rest, and inactivity as a consequence of congestive heart failure, stroke, chronic obstructive pulmonary disease, limb casting, and muscle unloading (i.e., microgravity). They were randomly divided into two groups. ES to the abdominal muscles was performed in one group in addition to the physical/occupational program (ES group; n = 10), while the physical/occupational program alone was performed in the other group as a control (Ctrl group; n = 10). The conventional physical and occupational therapeutic program included range of motion exercises, muscle strengthening exercises, ADL exercises, and so on, which were performed depending on the physical activity of each individual. The demographic and clinical parameters of the subjects at the beginning of the study are summarized in Table 1. There were no differences between the two groups for any of the items. All of the subjects underwent a standard physical/occupational therapy program twice a week during the study period. Owing to their poor physical condition on the day of examination (fever, blood high pressure, headache, arthralgia, and so on), a few subjects were not able to perform some of the tasks and examinations. The subjects were fully informed about the protocol, and provided informed consent to participate in the study. The study protocol was approved by the Institutional Review Board of Tohoku University Graduate School of Medicine and included the ethical rules for human experimentation stated in the Declaration of Helsinki.

Procedures

Bidirectional rectangular pulses of 0.2-ms duration were used and the frequency of the pulse train was 30 Hz in each direction. We placed the stimulation electrodes on both sides of the flank to stimulate more abdominal muscles. We could palpate the same contraction of the abdominal muscles located between the two electrodes in all of the subjects. The stimulation intensity was set at the maximum level just below the pain threshold, so that the subjects never perceived any unpleasant feelings. ES was applied to the abdominal muscles including the obliquus externus abdominis muscle (EO), obliquus internus abdominis muscle (IO), transversus abdominis muscle (Tr), and rectus abdominis muscle (Rc) on both sides. Surface electrodes of 5 × 10 cm (Lintec Co., Tokyo, Japan) were placed on the skin covering the muscles. A portable electrical stimulator (Nodoka; Lintec Co.) was used for ES. ES was performed for 15 min twice a day for 8 weeks (Fig. 1), separately from the physical/occupational therapy. At the beginning of the study and after 2 weeks (2W), 4 weeks (4W), and 8 weeks (8W), performance tests were carried out for all of the subjects to evaluate the changes in their physical functions. The abdominal muscles were evaluated by computed tomography and electromyography (EMG) at the beginning of the study and after 8W.

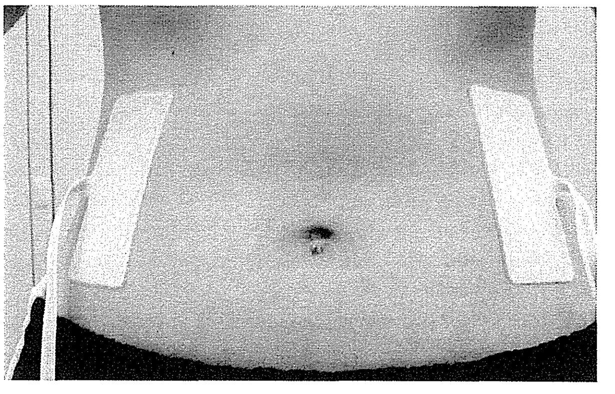

Fig. 1. Positions of the electrodes. The stimulation electrodes were placed on both sides of the flank. Bidirectional rectangular pulses of 0.2-ms duration were used and the frequency of the pulse train was 30 Hz in each direction. The bidirectional rectangular pulses stimulated both sides of the abdominal muscles equally. The stimulation intensity was set at the level just below the pain threshold so that the subjects never perceived any unpleasant feelings.

Performance tests

The number of trunk flexions (NTF) until the inferior angles of the scapulae were off the table for 30 sec in the supine position (Vera-Garcia et al. 2008), movement time required for sitting up (MSU) from the supine position as fast as possible (Bramell-Risberg et al. 2005), grip strengths of both sides (Nijland et al. 2010), movement time for sit-to-stand (STS) performed as soon as possible (Schenkman et al. 1996), walking speed (WS) for a 10-m distance with maximum effort, flexibility of the trunk (finger-toe distance: FTD) (Czaprowski et al. 2012), and ADL score (Barthel index: BI) were measured by physical therapists or occupational therapists who were unaware of the information of the two groups.

Measurement of the cross-sectional area

Previous studies reported there was a significant relationship between muscle strength and muscle cross-sectional area (CSA) (Dal Corso et al. 2007). We examined the morphological changes of the abdominal muscles using computed tomography. The CSA of the abdominal muscles were measured at the level of the umbilicus. The CSA of the Rc and flank muscles, including the EO, IO, and Tr, were calculated on each side by drawing the external margin of each muscle group. These methods were described previously (Mitsiopoulos et al. 1998; Goodpaster et al. 2000).

Measurement of electric muscle activity

Surface EMG was used to assess the physiological changes. The EMG signals for the Rc and EO were collected from bipolar surface electrodes on both sides. The electrodes on each muscle were placed at 3 cm lateral to the umbilicus for the Rc and at 10 cm lateral to the umbilicus for the EO according to a previously described method (Ng et al. 1998). EMG signals were continuously recorded during five trunk flexions. The raw EMG signals were sampled at 1,000 Hz and filtered to eliminate the electrical activities below 30 Hz (high pass) and above 100 Hz (low pass). Each EMG wave was integrated by calculating the area under the rectified curve during the middle 3 sec. Each integrated value was averaged to provide a parameter of the total amount of electric muscle activity (iEMG).

Statistical analysis

All data are presented as means ± S.D. Statistical analyses were performed using Wilcoxon's signed-rank test to compare within-group values before and after the study. The Mann-Whitney U test was used to compare unpaired data. Differences between the two groups were evaluated by two-way ANOVA using Stat Cel QC for Windows (OMMS Co., Saitama, Japan). Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Among the 20 subjects, one subject gave up because of lower abdominal pain, and some subjects could not be evaluated because of their poor physical condition (fever, headache, high blood pressure, low back pain, and so on). The numbers of subjects who were able to be evaluated are shown in Tables 2-5.

Changes in the functional parameters

The results of the performance tests for the two groups at each time point are shown in Tables 2 and 3. There were nonsignificant differences between the two groups for all of the functional parameters at the beginning of the study.

The NTF was significantly increased from 12.7 ± 4.4 to 14.2 ± 5.8 in the ES group after 4W ($p < 0.05$), while a nonsignificant change was observed in the Ctrl group (Fig. 2). One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, and arthralgia).

In the ES group, the MSU was significantly decreased from 23.5 ± 15.6 sec to 15.2 ± 12.9 sec after 4W ($p < 0.01$), while the Ctrl group showed a significant increase from 16.3 ± 14.3 sec to 22.6 ± 19.5 sec at 2W ($p < 0.05$) (Fig. 3). The significant decrease in the ES group from 23.5 ± 15.6 sec to 16.3 ± 14.3 sec after 8W was more prominent than that after 4W. One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, and arthralgia).

The STS was significantly increased from 29.2 ± 14.2 sec to 25.1 ± 12.5 sec in the ES group after 8W ($p < 0.05$), while a nonsignificant change from 35.4 ± 25.2 sec to 26.8

Table 2. Changes in motor performance parameters.

	ES group				Ctrl group			
	Start	2W	4W	8W	Start	2W	4W	8W
NTF (ES n = 8, Ctrl n = 9)	12.7 ± 4.4	14.2 ± 4.5	14.9 ± 5.8 *	15.6 ± 6.0 *	11.1 ± 7.5	10.5 ± 7.6	12.1 ± 7.2	10.5 ± 7.7
MSU (sec) (ES n = 8, Ctrl n = 9)	23.4 ± 15.6	15.6 ± 13.4	15.2 ± 12.9 **	12.7 ± 11.2 *	16.3 ± 14.3	22.6 ± 19.5 *	20.3 ± 16.4	15.4 ± 9.2
STS (sec) (ES n = 9, Ctrl n = 8)	29.2 ± 14.2	30.5 ± 17.1	25.1 ± 12.9	25.1 ± 12.5 *	35.4 ± 25.2	30.3 ± 16.9	25.1 ± 12.5	26.8 ± 17.9
WS (m / min) (ES n = 8, Ctrl n = 8)	13.0 ± 11.5	17.9 ± 17.2	17.0 ± 17.2	25.3 ± 18.1 *	12.1 ± 10.0	9.9 ± 8.9	10.1 ± 8.3	14.3 ± 6.0

Values are presented as the mean ± S.D.

*$p < 0.05$, **$p < 0.01$, significant difference from the value at the beginning of the study.

Start, at the beginning of the study; NTF, number of trunk flexions; MSU, movement time required for sitting up; STS, movement time required for sit-to-stand; WS, walking speed for a 10-m distance with maximum effort.

Table 3. Non-significant changes in motor performance parameters.

		ES group				Ctrl group			
		Start	2W	4W	8W	Start	2W	4W	8W
FTD (cm) (ES $n = 8$, Ctrl $n = 7$)		12.8 ± 9.6	13.2 ± 9.7	14.8 ± 11.5	16.5 ± 13.3	14.3 ± 9.8	17.3 ± 8.4	17.2 ± 9.4	14.9 ± 7.4
Grip strength (kg) (ES $n = 8$, Ctrl $n = 8$)	Rt	7.9 ± 5.3	8.3 ± 5.5	7.9 ± 6.1	6.6 ± 5.0	6.8 ± 5.2	7.0 ± 4.6	7.9 ± 6.1	6.9 ± 5.8
	Lt	15.1 ± 7.8	15.1 ± 6.7	16.5 ± 6.6	10.4 ± 5.6	11.4 ± 6.5	11.2 ± 5.1	16.5 ± 6.6	12.1 ± 6.0
BI (ES $n = 8$, Ctrl $n = 8$)		53.8 ± 20.8	54.4 ± 21.1	55.6 ± 19.3	57.1 ± 19.3	56.9 ± 21.4	56.9 ± 24.1	57.5 ± 21.5	57.1 ± 22.9

Values are presented as the mean ± S.D.
$*p < 0.05$, $**p < 0.01$, significant difference from the value at the beginning of the study.
Start, at the beginning of the study; FTD, finger-toe distance; BI, Barthel index.

Table 4. Changes in cross-sectional areas of the abdominal muscles.

Cross sectional area (mm^2)	ES group ($n = 8$)			Ctrl group ($n = 7$)		
	start	8W	p	start	8W	p
right Rc	11.7 ± 2.4	12.3 ± 1.8	< 0.05	13.6 ± 5.8	12.4 ± 4.5	.13
left Rc	11.1 ± 3.5	12.4 ± 2.8	.13	14.9 ± 7.0	14.0 ± 6.4	.23
right flank	50.4 ± 10.3	50.0 ± 8.6	.20	53.5 ± 14.4	47.0 ± 17.1	.06
left flank	48.1 ± 9.2	47.4 ± 14.6	.33	50.1 ± 16.6	42.3 ± 15.5	.08

Values are presented as the mean ± S.D.
Start, at the beginning of the study; Rc, rectus abdominis muscle.
The flank muscles include the obliquus externus abdominis muscle (EO), obliquus internus abdominis muscle (IO), and transversus abdominis muscle (Tr).

Table 5. Changes in the EMG activities of the abdominal muscles.

iEMG (mV)	ES group ($n = 7$)			Ctrl group ($n = 6$)		
	start	8W	p	start	8W	p
right Rc	14.1 ± 7.8	19.5 ± 12.8	.25	20.9 ± 19.6	13.7 ± 8.3	.17
Left Rc	14.6 ± 8.6	20.9 ± 10.0	< 0.05	27.3 ± 25.7	18.4 ± 17.3	< 0.05
right flank	7.4 ± 4.4	7.7 ± 4.5	.30	6.9 ± 3.3	6.4 ± 3.0	.46
left flank	8.0 ± 5.5	9.2 ± 4.8	.25	7.3 ± 2.1	6.6 ± 1.9	.46

Values are presented as the mean ± S.D.
Start, at the beginning of the study; Rc, rectal abdominal muscle.
The flank muscles include the obliquus externus abdominis muscle (EO), obliquus internus abdominis muscle (IO), and transversus abdominis muscle (Tr).

± 18.3 sec was observed in the Ctrl group during the study (Fig. 4). One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, and arthralgia).

Compared with the initial value, the WS was significantly increased from 13.0 ± 11.5 m/min to 25.3 ± 18.1 m/min in the ES group after 8W ($p < 0.05$), while a nonsignificant change was found in the Ctrl group during the study (Fig. 5). One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, low back pain, and arthralgia).

The FTD, bilateral grip strengths, and BI scores showed nonsignificant changes for both groups in Table 3. One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, low back pain, headache, and arthralgia).

Changes in CSA

The CSAs of the Rc and flank muscles did not differ

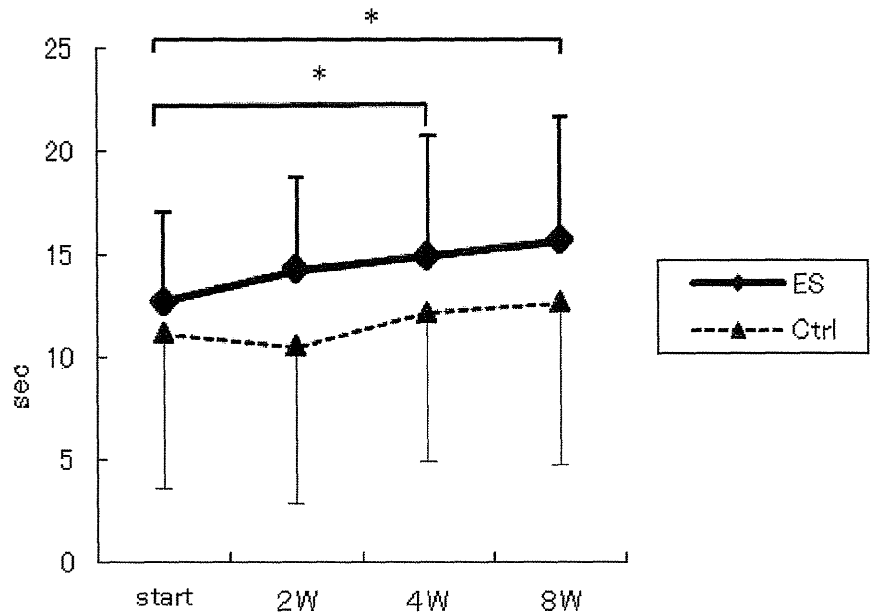

Fig. 2. Changes in the number of trunk flexions (NTF) after the intervention. The values in the ES group are significantly increased at 4W and 8W. *$p < 0.05$, significant difference from the value at the beginning of the study.

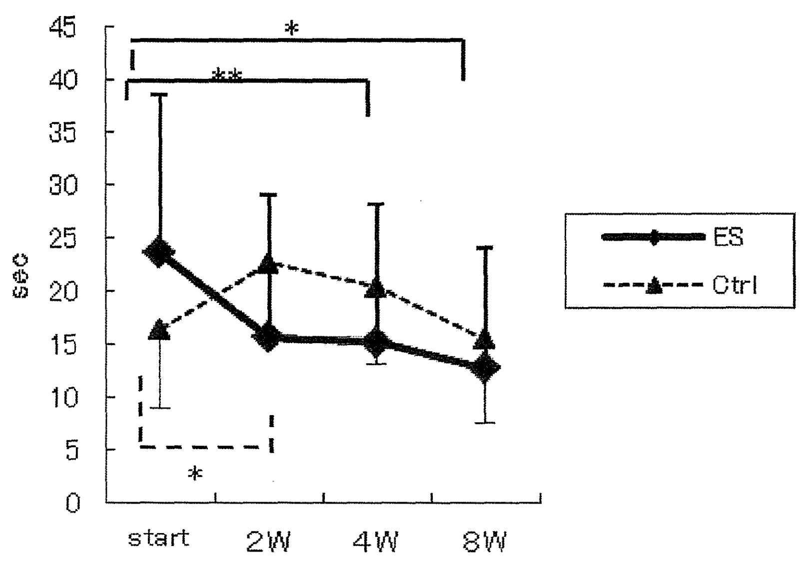

Fig. 3. Changes in the minimum time required for sitting up (MSU) after the intervention. The values in the ES group are significantly decreased at 4W and 8W, while the value in the Ctrl group shows a significant increase after 2W. *$p < 0.05$, **$p < 0.01$, significant difference from the value at the beginning of the study.

significantly between the two groups before the study. The CSA of the right Rc increased significantly from 11.7 ± 2.4 mm^2 to 12.3 ± 1.8 mm^2 in the ES group ($p < 0.05$), but decreased significantly from 13.6 ± 5.8 mm^2 to 12.4 ± 4.5 mm^2 in the Ctrl group in Table 4. Nonsignificant changes were noted in the other muscles in Table 4. One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, low back pain, and arthralgia).

Changes in iEMG

We only performed EMG for the Rc and EO. There were nonsignificant differences in the iEMG values for the Rc and EO before the study. The iEMG value for the left Rc increased significantly in the ES group after 8W ($p <$

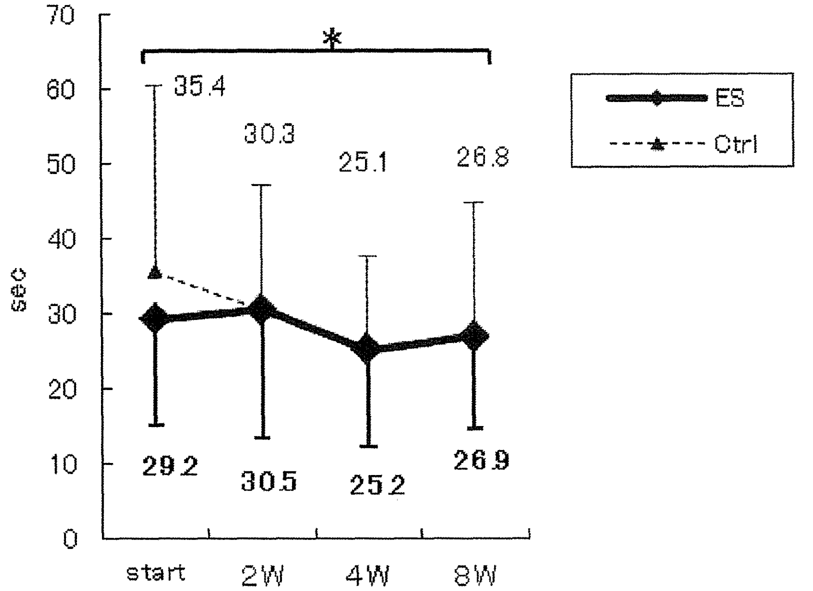

Fig. 4. Changes in the sit-to-stand time (STS) after the intervention. The value in the ES group shows a significant decrease after 8W. *$p < 0.05$, significant difference from the value at the beginning of the study.

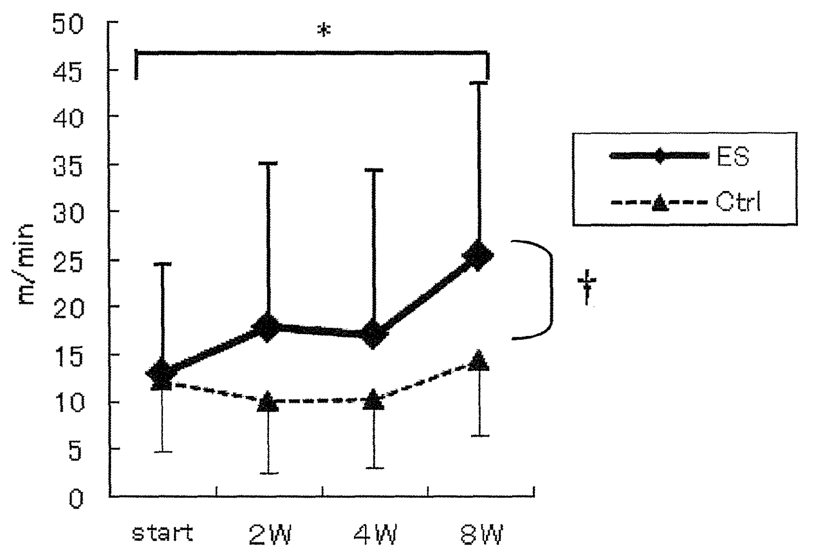

Fig. 5. Changes in the walking speed (WS) after the intervention. The value in the ES group shows a significant increase after 8W. *$p < 0.05$, significant difference from the value at the beginning of the study. †$p < 0.05$, significant difference between the two groups.

0.05), but decreased significantly in the Ctrl group ($p < 0.05$) in Table 5. Nonsignificant differences were found in the EO in Table 5. One subject gave up because of lower abdominal pain and some subjects could not be evaluated because of their poor physical condition (fever, high blood pressure, low back pain, headache, and arthralgia).

Discussion

The aim of this study was to examine the chronic effects of abdominal surface ES. We evaluated the effects of ES to the abdomen on selected functional parameters in inactive elderly subjects. In summary, ES to the abdomen may be able to change inactive elderly people to active elderly people.

In this study, we confirmed the improvement of several functional parameters such as the NTF, MSU, and WS in the ES group. Improvements were noted even after 4W. Specifically, we observed significant changes in the motor functions related to abdominal muscle activities, such as the NTF and MSU. Although the background mechanisms underlying such functional changes may be complicated, morphological and physiological changes to the abdominal muscles can be indicated.

First, the morphological changes to the abdominal muscles may provide increased muscle strength. The present results showed that the CSA of the right Rc was increased in the ES group after 8W. On the contrary, the CSA of the right Rc was significantly decreased in the Ctrl group. Although an increase in muscle volume does not necessarily mean an increase in muscle strength, there are several reports showing that muscle strength improves together with an increase in CSA in skeletal muscles (Ackermann et al. 2006; Dal Corso et al. 2007; Verdijk et al. 2009). Accordingly, it is possible that the ES group obtained more strength of the abdominal muscles than the Ctrl group, and the improvements in the NTF and MSU in the ES group may have been provided by such increases in the muscle strength.

Second, physiological changes in the abdominal muscles may be involved in an increase in muscle strength. The EMG measurements in the present study revealed that the iEMG value of the left Rc was significantly increased after the intervention in the ES group. This finding suggests that the number of neuromuscular units in the abdominal muscles was increased after ES. In other words, the strength of the abdominal muscles may have been improved by an increase in the number of neuromuscular units.

However, significant changes in the abdominal muscles from both the morphologic and physiological aspects were only found in the Rc. The EMG measurements were performed during tasks involving trunk flexion. When performing trunk flexion tasks, such as bowing, squatting down, sitting up, getting up, and so on, the subjects used muscle contraction of the Rc more than that of the flank muscles. Furthermore, it was reported that subjects used the Rc more than the flank muscles during ADL for breathing, excretion, posture adjustment, getting up, and so on (Workman et al. 2008). Therefore, the Rc may show more improvement in both the CSA and iEMG than the flank muscles.

In the ES group, a significant increase was observed in the CSA of the right Rc, while the iEMG value was significantly increased in the left Rc. The reason why such significant changes were only found on one side is unknown. Although we placed the stimulation electrodes on both sides of the flank in this study to stimulate more abdominal muscles (Rc, IO, EO, and Tr), we could palpate the same contraction of the abdominal muscles located between the two electrodes in all of the subjects. Indeed, the CSA of the left Rc was increased in five of eight subjects in the ES group. An increase in the iEMG of the right Rc was also observed in four of seven subjects. Therefore, the observation of significant differences on one side only may have been caused by the small number of subjects.

In general, increased muscle strength is provided by changes in neural factors (iEMG) and hypertrophy of muscle fibers (CSA) (Moritani and deVries 1980). Although it was recently reported that muscle strength is often brought about by hypertrophy through exercise (Folland and Williams 2007), an increase in muscle strength during the early period of training can be caused by an increase in the number of muscle fibers participating in the muscle contraction (Cormie et al. 2007). According to Moritani and de Vries (Moritani and de Vries 1980), both young and elderly groups showed an increase in muscle strength as a result of muscle-strengthening exercises for 8W, but muscle hypertrophy was only observed in the young group. Consequently, the authors concluded that the increase in muscle strength in the elderly group after exercise occurred through an increase in the number of neuromuscular units, rather than muscle hypertrophy. In the present study, we found early improvement of the NTF and MSU in the ES group, even after intervention for 4W. Eriksson and Häggmark (Eriksson and Häggmark 1979) reported that ES required 8W to bring about muscle hypertrophy. Therefore, it is indicated that the early improvement of motor function after intervention with ES was caused by physiological changes in the abdominal muscles, similar to the case for physical exercise.

The WS and STS were significantly changed after 8W in the ES group, but not in the Ctrl group. From the viewpoint of normal development, standing and walking can be realized on the basis of motions on a bed, such as the MSU. Therefore, it is valid to consider that the late improvements in the WS and STS followed the early improvements in the NTF and MSU, reflecting motions on a bed. The increase in abdominal muscle strength obtained during the first 4 weeks after the initiation of ES may contribute to the subsequent facilitation of standing and walking. On the other hand, the grip strengths and FTD showed no changes during the study period. Grip strength is an indicator of muscle strength in the whole body, while the FTD reflects whole body flexibility. Furthermore, no change was found in the BI score despite the improvements in the WS and STS in the ES group. Nevertheless, it is said that a WS of more 20 m/min, similar to the result in the ES group, may be associated with a higher probability of being independent in ADL (Vermeulen et al. 2011). Consequently, the BI as a scale for assessing ADL is too inaccurate to evaluate precise changes. The findings for the grip strengths and FTD suggest that global changes relating to the whole body function did not occur in the present study. Therefore, the effects of ES to the abdomen are limited to the local functions executed by the abdominal muscles.

The abdominal muscles have important roles for not only trunk function, but also the whole body motor function, and contribute to breathing, speaking, and defecation. However, the strength of these muscles declines easily with the process of aging. It has been reported that muscle atrophy is based on the aging characteristics of type II fibers

(Lexell et al. 1988; Porter et al. 1995; Kararizou et al. 2009) and decreased acceleration of muscle power (Saini et al. 2009). Therefore, reinforcement of type II fibers rather than type I fibers is required for the elderly (Takano et al. 2010). However, effective rehabilitation programs for inactive elderly people with limited mobility, who spend most of their time on a bed, are limited to exercises involving low loads or stretching, and therefore positive effects on muscle strength cannot be expected with such programs. Several investigators have examined the responses of muscle strength to repeated sessions of ES, and reported increased strength of the skeletal muscles, improved muscle contraction in patients after knee surgery, and reduced atrophy (Kondrashin et al. 1975; Eriksson and Haggmark 1979; Romero et al. 1982; Currier and Mann 1983). These findings indicate that ES can prevent and improve disuse owing to immobilization. ES also produces immediate changes in the motor units, by increasing the recruitment number and action potential velocity and establishing the selective recruitment of type II fibers (Montes Molina et al. 1997). The safety and efficacy of ES to the skeletal muscles have recently been confirmed, even for heart failure patients (Dobsak et al. 2006). Therefore, ES to the abdominal area has good potential for improving the motor function of inactive elderly people through abdominal muscle strengthening.

Problems specific for ES to the abdomen include the potential to cause local skin burns or irritation, electrode sensitivity, autonomic dysreflexia, and problems with use over healing wounds (Gorman 2000). However, we could not confirm such side effects in this study.

Some potential limitations of our study should be considered. First, this study involved a relatively small number of subjects. Second, the period of the study was too short to reveal the long-term effects of ES. Future studies with larger numbers of subjects and longer observation times are necessary to clarify the precise and long-term effects of ES to the abdomen.

Conclusion

This study is the first randomized trial aiming to show the effectiveness of surface ES to the abdomen for inactive elderly people. The present results revealed functional improvements in the ES group. We conclude that the surface ES to the abdomen should be taken into consideration as one of the rehabilitation programs for inactive elderly people.

Conflict of Interest

The authors declare no conflict of interest.

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SCIENTIFIC RESEARCH ARTICLE

Functional Changes in the Care-needing Elderly after Surface Electrical Stimulation to the Abdomen

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ABSTRACT. Background: Strength of the trunk muscles is a key component of motor control, but it declines easily with the process of aging and/or disuse. Objectives: To investigate the effects of surface electrical stimulation (ES) to the abdominal muscles and the motor performance for care-needing elderly. Design: Controlled trial. Participants: Twenty-one elderly people (60–90 years) with care-needing, who were admitted to a nursing home or hospital for daily care and their score of Barthel Index had been stable from 55.3 ± 19.4 to 55.3 ± 19.8 during 3 months before intervention. Intervention: Common physical/occupational therapy was performed for all of the subjects during the study period. ES was applied to the abdomen of 15 subjects (ES group) twice a day for 8 weeks. Measurements: Functional measurements were performed before and after beginning of the study with some parameters as follows: grip strength; movement time for sitting up (MSU); maximum walking speed (WS). Results: No significant differences between two groups were noted for characteristics at baseline. In the ES group, MSU (from 21.1 ± 21.4 to 10.4 ± 8.3 sec) significantly improved ($p < 0.05$) after 8weeks. WS (from 17.7 ± 12.1 to 24.3 ± 15.3 m/min) were also improved ($p < 0.05$) after 8 weeks. Limitations: A limitation of this study was the small sample size. Conclusions: ES to the abdominal area has the potential to improve motor function in the care-needing elderly through reinforcement of the abdominal muscles.

Key words: abdominal muscles, care-needing elderly, surface electrical stimulation

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In recent years, with the increasing number of elderly people in Japan, preventive care[1] has become of considerable importance. Loss of muscle strength with aging has been suggested to be one of the main factors causing functional limitations in the activities of daily living, such as rising from a chair or climbing up and down stairs[2–4]. The strength of the trunk muscles declines easily with the process of aging and/or disuse, and these muscles have important roles not only for trunk function but also for the whole body motor function[5]. The subscore of the functional independence measures relating to locomotion-transfers in stroke subjects at discharge was reported to be positively correlated with trunk muscle torque values, except for isometric extension[6].

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Machine training, aerobics and other activities are recommended for health promotion in the elderly, but there are few programs available for bedridden elderly patients those who deteriorated of the will to strengthen their weak abdominal muscles. Some methods of physical therapy, including electrical stimulation (ES), have been used for muscle strengthening. In particular, ES has been adopted to improve not only muscle atrophy but also impaired motor function in hemiplegic patients and spinal cord-injured patients since the 1980s, and its safety and effectiveness have been confirmed[7–11]. In the present study, we applied surface ES to the abdominal muscles of care-needing elderly people and investigated its effects in motor performance.

Methods

Subjects and Overall Design

This prospective randomized study was carried out in

Table 1. Demographic and clinical parameters of the subjects

	ES group (n=15)	Ctrl group (n=6)	P
	77.9±8.9	73.5±8.2	0.19
Height (cm)	150.2±9.9	152.3±12.4	0.97
Weight (kg)	54.9±12.3	48.6±11.1	0.17
BMI	24.1 ±4.1	21.0±3.9	0.17

Values are presented as the mean ±SD. No significant differences beween two groups were noted for characteristics at baseline. BMI: body mass index.

21 elderly people (10 males and 11 females) who were admitted to an institute or a hospital for nursing care. The subjects were prolonged decrease in muscle contractile activity, such as that in a sedentary lifestyle, during periods of prolonged bed rest, inactivity as a consequence of congestive heart failure, stroke, COPD, limb casting and muscle unloading (i.e., microgravity). Although continuous intervention with common physical therapy was performed, their score of Barthel Index had been stable from 55.3 ± 19.4 to 55.3 ± 19.8 during 3 months before the start of the study owing to chronic disuse and they could walk with using the device (cane, walker, orhosis and so on) They had spent the major part of each day in their bedroom. The subjects were divided into two groups. Before study started, the subjects were fully informed about the protocol, and provided informed consent to participate in the study. The study protocol was approved by the Institutional Review Board of Tohoku University Graduate School of Medicine including the ethical rules for human experimentation that are stated in the Declaration of Helsinki.

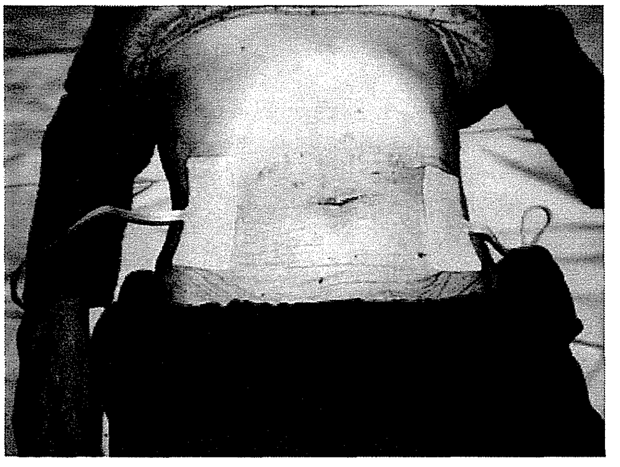

Fig. 1. Positions of the electrodes. The stimulation electrodes were placed on the trunk over the abdominal oblique muscles.

Participants were randomly assigned to 2 groups. ES to the abdominal muscles was performed in one group in addition to the physical/occupational program (ES group; nine males and six females n = 15), while the physical/occupational program that depends on personal physical ability, such as ROM ex, muscle-strength ex and so on, alone was performed in the other group as a control (Ctrl group; four males and two females n = 6). Randomization was carried out electrically by a physical therapist who was not informed about this study. The demographic and clinical parameters of the subjects at the beginning of the study are summarized in (Table 1). There were no differences between the two groups for any of the items. All of the subjects underwent a standard physical/occupational therapy program twice a week during the study period.

Procedures

ES was applied to the abdominal muscles including the obliquus externus abdominis muscle (EO), obliquus internus abdominis muscle (IO), transversus abdominis muscle (Tr) and rectus abdominis muscle (Rc) on both sides. Surface electrodes of 5 × 10 cm (Lintec Co., Tokyo, Japan) were placed on the skin covering the muscles, except for the Rc, which was located below the inferior border of the 12th rib. A portable electrical stimulator (Nodoka; Lintec Co.) was used for ES. Cyclic stimulation with 10 sec ON and 5 sec OFF was performed for 15 min twice a day for 8 weeks (Fig. 1), separately from the physical/occupational therapy. Bidirectional rectangular pulses with 0.2 msec duration were used and frequency of the pulse train was 30 Hz in each direction. Thus we confirmed that EO, IO, Tr and Rc were stimulated by this method to a normal adult before the study start. The stimulation intensity was set at the maximum level just below the pain threshold so that the subjects never perceived any unpleasant feelings. At the beginning of the study and after 8 weeks (8W), performance tests were carried out for all of the subjects to evaluate the changes in their physical functions.

Performance Tests

Movement time required for sitting up (MSU)

Rising from lying supine on a physiotherapy bench to sitting on the edge and rising to sitting were measured[12]. Two trials were performed successively and the fastest time

Table 2. Changes in the Parameters of Motor Performance

	ES group (n=15)			Ctrl group (n=6)		
	before	After 8W	P	before	After 8W	P
MSU(s)	21.1±21.4	10.4±8.3*	0.029	18.0±17.3	17.1±17.2	0.74
WS(m/min)	17.7±24.3	24.3±15.3*	0.017	9.3±10.3	7.2±7.7	0.77
grip (kg)	19.4±9.7	21.1±10.7	0.20	11.2±5.5	12.1±5.4	0.10

Values are presented as the mean ±SD. No significant differences between two groups were noted for characteristics at baseline. $*P < 0.05$, significant difference from the value at the beginning of the study.
MSU: Movement time required for sitting up
WS: Walking speed for 10m distance with maximum effort

was used for the calculations. The bench used was a semi-firm bench with a plastic-covered surface, 200 ×100 cm with a height of 55 cm[12].

Grip strengths

For grip strength measurements, a digital hand dynamometer (Model T.K.K.5401, Takei Kogyou, Inc., Tokyo) was used. The test was administered by a trained physical or occupational therapist and highest of two measures in the dominant hand was used in the analysis[13].

Walking speed for 10 m distance with maximum effort (WS)

The patient began to walk as quickly as possible 3 m before the starting line, and the timing commenced when the patient's swing leg crossed the starting line and ended when the patient's swing leg passed over the finish line by physical therapists or occupational therapists that were unaware of the information of the groups. Two trials were performed successively and the fastest time was used for the calculations[14].

Measurement of the Cross-sectional Area

We examined the morphologic changes of the abdominal muscles using Computed Tomography (CT) in only one subject by a Radiological technologist. The cross-sectional area of the abdominal muscles was measured at the level of the umbilicus. The level of the middle of vertebra L3 was located using the CT scout image (Althen 2005) as a guide, and eFilm software (Image J; New York) employed to estimate the area using an ellipse draw and analysis tool. The results generated by the draw and analysis tool are indicated on the image. The cross-sectional areas of the Rc and flank muscles including the EO, IO and Tr were calculated on each side by drawing the external margin of each muscle group. These methods were described previously[15].

Statistical Analysis

All data are presented as means ± SD. Statistical analysis was performed using Shapiro-wilk test and Levene test, then the paired Student t test to compare within-group values before and after the intervention and the unpaired Student t test was used to compare unpaired data between both groups, and using Stat Cel QC for Windows. Values of $P < 0.05$ were considered to indicate statistical significance.

Results

Changes in the Functional Parameters

No significant differences between two groups were noted for characteristics at baseline. In the ES group, MSU was significantly decreased after 8W ($P < 0.05$), while non-significant change was observed in the Ctrl group. (Table 2). Compared with the initial value, WS was significantly increased ($P < 0.05$) in the ES group, while non-significant change was found in the Ctrl group during the study (Table 2).

The grip strength which indicates whole body function showed non-significant changes in both groups.

Changes in the cross sectional area of abdominal muscles

Though abdominal CT was examined by a Radiological technologist only in one subject, the increase of abdominal muscle volume was confirmed in this case (Fig. 2). The increase of abdominal muscle volume from 952 mm^2 to 1329 mm^2 in this case could indicate the local effects of ES.

Discussion

The aim of this study was to examine the effects of abdominal surface ES on the functions of a limited part of the body and the whole body. We evaluated the effects of ES to the abdomen on selected functional parameters in care-needing elderly subjects. In summary, ES to the abdomen may be able to change care-needing elderly people to active.

In this study, we confirmed the improvement of several functional parameters such as the MSU and WS in the ES group. The improvements were confirmed after 8W. Although the background mechanism underlying such functional changes will be complicated, certain factors can be indicated.

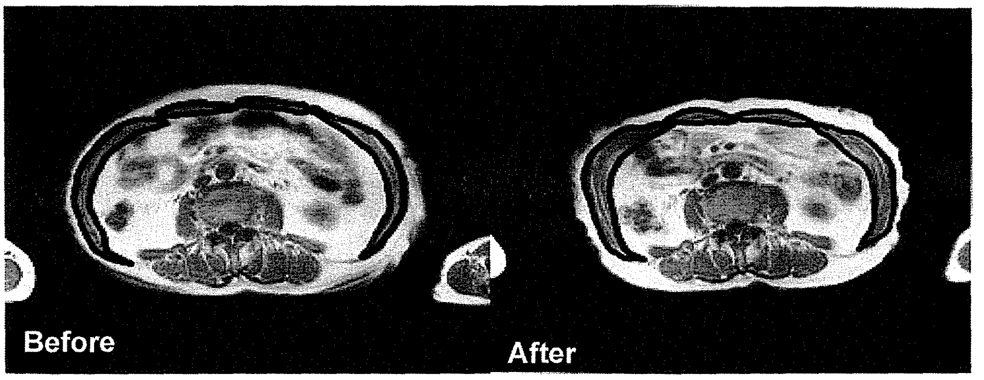

Fig. 2. A change in the abdominal CT was examined only in one subject by Computed tomography. The increase of abdominal muscle volume from 952 mm^2 to 1329 mm^2 in this case could indicate the local effects of ES.

First, morphologic changes such as (Fig. 2) to the abdominal muscles may provide increased muscle strength. There are several reports showing that muscle strength improves together with an increase in the cross-sectional area in skeletal muscles[16–18]. Accordingly, it is possible that the ES group obtained more strength of the abdominal muscles than the Ctrl group and the improvements in the MSU in the ES group may have been provided by such increases in the muscle strength.

Second, physiologic changes in the abdominal muscles may be involved in an increase in muscle strength. This finding suggests that the number of neuromuscular units in the abdominal muscles was increased after ES. In other words, the strength of the abdominal muscles may have been improved by an increase in the number of neuromuscular units.

In general, increased muscle strength is provided by changes in neural factors and hypertrophy of the muscle fibers. Although it was recently reported that muscle strength is often brought about by hypertrophy through exercise[19], an increase in muscle strength during the early period of training can be caused by an increase in the number of muscle fibers participating in the muscle contraction[20]. According to Moritani and de Vries,[21] both young and elderly groups showed an increase in muscle strength as a result of muscle-strengthening exercises for 8W, but muscle hypertrophy was only observed in the young group. Consequently, the authors concluded that the increase in muscle strength in the elderly group after exercise occurred through an increase in the number of neuromuscular units rather than muscle hypertrophy. In the present study, we found improvement of the MSU in the ES group. Eriksson *et al.*[22] reported that ES required 8W to bring about muscle hypertrophy. Therefore, it is suspected that the early improvement of motor function after intervention with ES was caused by physiologic changes in the abdominal muscles, similar to the case for physical exercise.

The WS was significantly changed after 8W in the ES group, but not in the Ctrl group. From the viewpoint of normal development, standing and walking can be realized on the basis of motions on a bed, such as sitting up. Therefore, it is valid to consider that the late improvements in the WS followed the early improvements in the MSU, reflecting motions on a bed. The increase in the abdominal muscle strength obtained after the initiation of ES may contribute to subsequent facilitation of standing and walking. On the other hand, the grip strengths showed no changes during the study period. Grip strength is an indicator of muscle strength in the whole body. The findings for the grip strengths suggested that global changes relating to the whole body function did not occur in the present study. Therefore, the effects of ES to the abdomen are limited to the local functions executed by the abdominal muscles.

The trunk muscles have important roles not only for trunk function but also for whole body motor function, and contribute to breathing, utterance and defecation. However, the strength of these muscles declines easily with the process of aging. It has been reported that muscle atrophy is based on the aging characteristics of type II fibers[23–25] and decreased acceleration of muscle power[26]. Therefore, reinforcement of type II fibers rather than type I fibers is required for the elderly[27]. However, effective rehabilitation programs for care-needing elderly people with limited mobility, who spend most of their time on a bed, are limited to exercises involving low loads or stretching, and therefore positive effects on muscle strength cannot be expected with such programs. Several investigators have examined the responses of muscle strength to repeated sessions of ES, and reported increased strength of the skeletal muscles, improved muscle contraction in patients after knee surgery and reduced atrophy[22, 28–30]. These findings indicate that ES can prevent and improve disuse owing to immobilization. ES also produces immediate changes in the motor units, by increasing the recruitment number and action potential velocity and establishing the selective recruitment of type II fibers[31]. The safety and efficacy of ES to the skeletal muscles have recently been confirmed, even for heart failure patients[32]. Therefore, ES to the abdominal area has good potential for improving the motor function of care-needing elderly people through abdominal muscle strengthening.

Some potential limitations of our study should be considered. First, this study involved a relatively small number of subjects. Second, we did not measure muscle activities and cross sectional area of the abdominal muscles. Futures studies with more than thirty numbers of subjects (using G*power for Windows) and muscle activities and cross sectional area of abdomen are necessary to clarify the precise and long-term effects of ES to the abdomen.

Conclusions

This study is the first controlled trial aiming to show the effectiveness of surface ES to the abdomen for care-needing elderly people. The present results revealed functional improvements in the ES group. We conclude that the results of this study are unique and that surface ES to the abdomen should be taken into consideration as one of the rehabilitation programs for the care-needing elderly.

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Disorder of fatty acid metabolism in the kidney of PAN-induced nephrotic rats

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Muroya Y, Ito O, Rong R, Takashima K, Ito D, Cao P, Nakamura Y, Joh K, Kohzuki M. Disorder of fatty acid metabolism in the kidney of PAN-induced nephrotic rats. *Am J Physiol Renal Physiol* 303: F1070–F1079, 2012. First published August 8, 2012; doi:10.1152/ajprenal.00365.2011.—Proteinuria is considered to play an essential role in the progression of tubulointerstitial damage, which causes end-stage renal disease. Fatty acid-binding albumins are filtered through glomeruli and reabsorbed into proximal tubular epithelial cells (PTECs). However, the role of fatty acid metabolism associated with albuminuria in the development of tubulointerstitial damage remains unclear. Thus, the present study was designed to determine the changes of fatty acid metabolism in the nephrotic kidney. To induce nephrotic syndrome, Sprague-Dawley rats (SDRs) and Nagase analbuminemic rats (NARs) with inherited hypoalbuminemia were treated with a single injection of puromycin aminonucleoside (PAN). In SDRs, PAN treatment induced massive proteinuria and albuminuria and caused tubular damage, apoptosis, and lipid accumulation in PTECs. Among the enzymes of fatty acid metabolism, expressions of medium-chain acyl-CoA dehydrogenase (MCAD) and cytochrome *P*-450 (CYP)4A significantly decreased in PTECs of PAN-treated SDRs. Expressions of peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α and estrogen-related receptor (ERR)α also significantly decreased, without changes in the expression of PPAR-α. In NARs, PAN treatment induced proteinuria but not albuminuria and did not cause tubular damage, apoptosis, or lipid accumulation. Expressions of MCAD, PGC-1α, or ERRα did not change in the kidney cortex of PAN-treated NARs, but the expression of CYP4A significantly decreased. These results indicate that massive albuminuria causes tubular damage and lipid accumulation with the reduction of MCAD, CYP4A, PGC-1α, and ERRα in PTECs.

albuminuria; lipid accumulation; lipotoxicity; fatty acid metabolism; proximal tubular epithelial cells; puromycin aminonucleoside

PROTEINURIA is considered to play an essential role in the progression of tubulointerstitial damage, which is a common pathway to end-stage renal disease (28, 30). Fatty acids (FAs) bound to serum albumin (7) are filtered through glomeruli and reabsorbed into proximal tubular epithelial cells (PTECs) via endocytosis. FAs were markedly reabsorbed and accumulated in PTECs of patients and experimental animals with nephrosis (16, 32). FAs also play a role in the development of tubulointerstitial damage as lipotoxicity (11, 18, 39, 46), which induces apoptosis by stimulating inflammation and oxidative stress.

The intracellular metabolism of FAs in PTECs is regulated by several steps: transport, synthesis, and oxidation of FAs. FA-binding protein (FABP) delivers FAs to intracellular sites of utilization. FA synthesis is catalyzed through FA synthase (FAS) and acetyl-CoA carboxylase (ACC). FAs undergo oxidation in mitochondria, the peroxisome, and the microsome. Medium-chain FAs (6–12 carbon chains) freely diffuse into mitochondria, and long-chain FAs are transported into mitochondria by carnitine palmitoyltransferase (CPT)-I. Acyl-CoA dehydrogenases are mitochondrial enzymes catalyzing the initial step in each cycle of FA β-oxidation and are categorized into distinct groups, such as very long-chain acyl-CoA dehydrogenase (VLCAD), long-chain acyl-CoA dehydrogenase (LCAD), medium-chain acyl-CoA dehydrogenase (MCAD), and short-chain acyl-CoA dehydrogenase (SCAD). Besides fatty acid β-oxidation in mitochondria, acyl-CoA oxidase (ACOX) is a peroxisomal enzyme breaking very long-chain FAs (20 or more carbon chains) down to a manageable size. Cytochrome *P*-450 (CYP)4A is a microsomal enzyme relating FA ω-oxidation of medium-chain and long-chain FAs.

Sterol regulatory element-binding proteins (SREBPs) serve as the master regulators of cellular lipid synthesis; SREBP-1 regulates fatty acid synthesis, and SREBP-2 regulates cholesterol synthesis (3). Peroxisome proliferator-activated receptor (PPAR)-α is essential for the maintenance of FA metabolism (1, 13, 44) as well as the function of PTECs (13). In addition to PPAR-α, PPAR-γ coactivator (PGC)-1α regulates FA metabolism and mitochondrial biogenesis (8, 33, 37). PGC-1α upregulates the expression and activity of the orphan nuclear receptor estrogen-related receptor (ERR)α (34), which is a transcriptional regulator of the MCAD gene (36).

Previous studies have reported disorders of FA metabolism in several kidney diseases. Lipid contents are increased in the kidney of chronic nephrotic rats (16). Lipid accumulation was also found in the remnant kidney of 5/6 nephrectomized rats accompanied by the upregulation of FAS and ACC expression and downregulation of FABP, CPT-I, ACOX, SREBP, and PPAR-α expression (4, 15). MCAD expression was downregulated in the mouse kidney with cisplatin-induced acute renal failure and ischemia-reperfusion injury (20, 21, 26). ACOX and CYP4A expressions were downregulated in the rat kidney with ischemia-reperfusion injury (27). Although the described animal models with kidney diseases have various disorders of FA metabolism, the role of FA metabolism associated with albuminuria in the development of tubulointerstitial damage remains unclear. Thus, the present study was designed to determine the changes of FA metabolism in the nephrotic kidney of Sprague-Dawley rats (SDRs) and to further compare them with those of Nagase analbuminemic rats (NARs) with inherited hypoalbuminemia.

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MATERIALS AND METHODS

Animals. Six-week-old male SDRs and NARs weighing 160–180 g were obtained from SLC (Shizuoka, Japan). Rats were housed in an animal care facility at Tohoku University School of Medicine and had free access to standard laboratory chow and water while housed under controlled environmental conditions (12:12-h light-dark cycle and 24°C room temperature). All protocols involving rats were reviewed and received prior approval by the Animal Welfare Committee of Tohoku University.

Rats were randomly divided into four groups: the control SDR group (n = 24), the puromycin aminonucleoside (PAN)-treated SDR (PAN-SDR) group (n = 24), the control NAR group (n = 6), or the PAN-treated NAR (PAN-NAR) group (n = 6). Vehicle (0.9% NaCl) or PAN (Sigma-Aldrich) at a dose of 100 mg/kg (35) was injected intravenously in each group. Rats were placed in individual metabolic cages (TOYO-RIKO, Tokyo, Japan) for 24 h, and urine samples were collected. Blood samples were collected by decapitation under anesthesia with pentobarbital sodium (50 mg/kg ip) on the 14th day after PAN treatment. Urine and blood samples were centrifuged for 5 min at 1,500 rpm, separated from the sediments, and stored at −80°C.

Measurement of biochemical parameters. Plasma total protein, albumin, total cholesterol, triglyceride, free FAs, urea nitrogen, and creatinine were measured by a standard autoanalysis technique. Urinary protein, *N*-acetyl-β-D-glucosaminidase (NAG), and 8-hydroxydeoxyguanosine (8-OHdG) were measured by Mitsubishi Chemical Medicine (Tokyo, Japan). Urinary albumin was determined by SRL (Tokyo, Japan).

Preparation of kidney and glomerular homogenates. The kidney was quickly removed after decapitation and sectioned into the cortex and medulla on the 14th day after PAN treatment. The kidney cortex was homogenized in 100 mmol/l potassium buffer (pH 7.25) containing 30% glycerol, 1 mmol/l DTT, and 0.1 mmol/l PMSF. The homogenate was centrifuged at 3,000 g for 5min, and the supernatant was collected.

The glomeruli were isolated using a rapid sieving technique as previously described (10). The kidney was flushed with 10 ml of a cold HEPES-buffered solution (4°C) containing 135 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM KH_2PO_4, 5.5 mM glucose, and 10 mM HEPES (pH 7.4). The kidney cortex was forced through a 180-μm stainless steel sieve using the plunger of a 30-ml syringe. The material passing through the sieve was passed through a 100-μm nylon sieve and collected on a 70-μm nylon sieve. After being rinsed with the HEPES-buffered solution, this fraction was enriched with the glomeruli (>95%). The glomeruli were then collected, resuspended in 200 μl of the potassium buffer, and homogenized by sonication for 15 s at moderate power. The homogenate was centrifuged at 3,000 g for 5 min, and the supernatant was collected.

Homogenate samples of the kidney cortex and glomeruli were snap frozen in liquid nitrogen and stored at −80°C. The protein concentration of the samples was measured using the Bradford method with bovine γ-globulin as the standard.

Western blot analysis. Proteins of the kidney homogenate (20 μg) were separated by electrophoresis on a 10 × 20-cm, 8.5% SDS-polyacrylamide gel (using SDS-PAGE) for 40 min at 120 V. Proteins were transferred electrophoretically to a nitrocellulose membrane at 100 V in a transfer buffer consisting of 25 mmol/l Tris·HCl, 192 mmol/l glycine, and 20% methanol for 1 h at 4°C. The membrane was blocked overnight at 4°C by an immersion into a buffer containing 10 mmol/l Tris·HCl, 150 mmol/l NaCl, and 0.08% Tween 20 (TBST buffer) with 10% nonfat dry milk. The membrane was then incubated for 2 h with primary antibodies raised against nephrin, dendrin, caspase 3, FAS, ACC, liver-type (L-)FABP, CPT-I, VLCAD, LCAD, MCAD, SCAD, ACOX, PPAR-α, PGC-1α, and ERRα (Santa Cruz Biotechnology) or CYP4A (Nosan, Tokyo, Japan). The membrane was rinsed several times with TBST buffer and then incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) for 1 h. After being washed in TBST, immunoblots were developed using an enhanced chemiluminescence kit (Super Signal, Thermo Fisher Scientific). Relative intensities were quantified using ImageJ software (version 1.44, National Institutes of Health). Band intensities for each protein were normalized to those for β-actin as an internal standard, and the band intensity in the control SDR group was assigned a value of 1. Immunoblot specificities of kidney homogenates were verified by blockade with corresponding control peptides for FAS, ACC, L-FABP, CPT-I, ACOX, PPAR-α, PGC-1α, and ERRα (Santa Cruz Biotechnology) or full-length recombinant proteins for human nephrin, caspase 3 (Novus), acyl-CoA dehydrogenases (Abnova, Taipei, Taiwan), and CYP4A11 (Becton Dickinson).

Tissue lipid contents. Total lipids were extracted from 100 mg of the kidney cortex by a previously described method (6). Briefly, samples were homogenized in 6 ml of chloroform-methanol (2:1). The mixture stood for 1 h, after which 1.5 ml water was added, and the mixture was centrifuged at 2,000 g for 10 min. The organic phase was evaporated to dryness and taken up in chloroform; 100 μl of this organic phase were solubilized by adding 1 ml 2-propanol, and free FA and triglyceride contents were determined using lipid assay kits (NEFA C-Test Wako and Triglyceride E-Test Wako) from Wako Pure Chemical (Osaka, Japan). Data are expressed as the amount of the lipids per 1 g of the kidney cortex mass.

Histopathology (electron microscopy), immunohistochemistry, and oil red O stain. For electron microscopy, small blocks of the kidney cortex were fixed with 2% glutaraldehyde and postfixed in 1% OsO_4. Samples were dehydrated in graded ethanol and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by electron microscopy (JEM-100SX, JEOL).

For immunohistochemical analysis, kidney tissues were fixed with 10% paraformaldehyde and embedded in paraffin. Paraffin sections (3 μm thick) were deparaffinized in xylene and ethanol and rinsed in PBS. To block endogenous peroxidase activity, rehydrated sections were treated with 0.3% H_2O_2 in absolute ethanol for 30 min and then processed for immunostaining with antibodies against caspase 3, MCAD, PGC-1α, and ERRα (1:500, respectively, Santa Cruz Biotechnology) or CYP4A (1:500, Nosan) with Histofine Simple Stain Max PO kits (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. After being washed with PBS, a chromogen solution, consisting diaminobenzidine and H_2O_2, was applied to the sections. Slides were counterstained with hematoxylin for 1 min.

For oil red O staining of tissue lipids, kidney tissues were fixed with 10% paraformaldehyde and made into frozen sections, and a portion was then stained with oil red O. Sections (6 μm thick) were rinsed in distilled water, rinsed in 60% isopropanol for 1 min, stained for 15 min in the oil red O working solution (Muto Pure Chemicals, Tokyo, Japan), rinsed again for 1 min in 60% isopropanol, and then returned to distilled water. Slides were counterstained with hematoxylin for 1 min.

Statistical analysis. Data are presented as means ± SE. Data were analyzed by repeated-measures ANOVA followed by a Tukey test for multiple comparisons among the groups. Paired data were analyzed by a Student's t-test. P values of <0.05 were considered to indicate statistical significance.

RESULTS

General data. A single intravenous injection of PAN induced massive proteinuria in SDRs. Time courses of urinary protein excretion in the control SDR and PAN-SDR groups are shown in Fig. 1*A*. Urinary protein excretion increased after the 3rd day after PAN treatment, continued to increase to a maximum level until the 14th day, and normalized on the 28th day. Therefore, the later experiments provided the data obtained on the 14th day after PAN treatment. Urinary protein

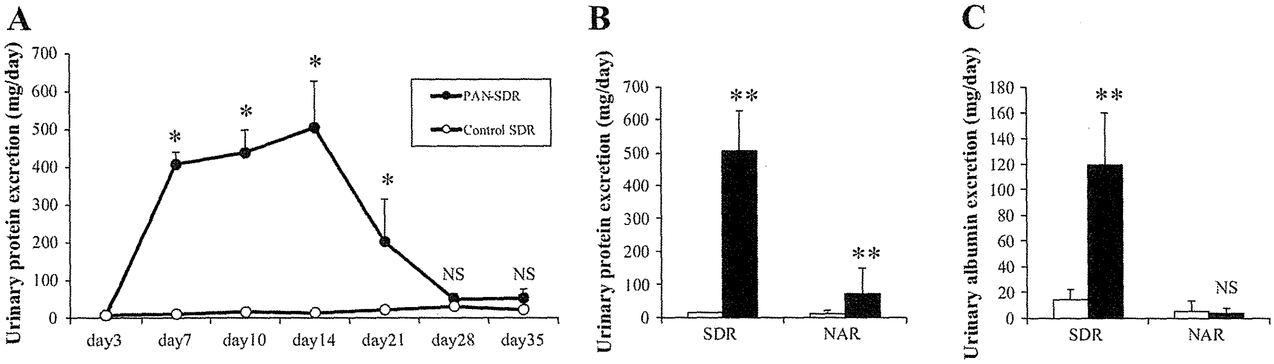

Fig. 1. Effects of puromycin aminonucleoside (PAN) treatment on urinary protein excretion in Sprague-Dawley rats (SDRs) and Nagase analbuminemic rats (NARs). The time courses of urinary protein excretion (*A*) were compared between the control SDR group and the PAN-treated SDR (PAN-SDR) group. Urinary protein excretion (*B*) and albumin excretion (*C*) were compared with the control group and PAN-treated group of either SDRs or NARs on the 14th day after PAN treatment. Data are presented as means ± SE; $n = 6$ rats/group. *$P < 0.05$ and **$P < 0.01$ compared with the corresponding control group. NS, no significant differences compared with the corresponding control group.

excretion and albumin excretion were significantly higher in the PAN-SDR group than in the control SDR group (Fig. 1, *B* and *C*). In contrast to the SDR group, urinary protein excretion was significantly higher in the PAN-NAR group than in the control NAR group (Fig. 1*B*), but urinary albumin excretion was barely detected and not significantly different between the control NAR group and PAN-NAR group (Fig. 1*C*).

The effects of PAN treatment on plasma parameters in SDRs and NARs are shown in Table 1. Plasma total protein and albumin were significantly lower in the control NAR group than in the control SDR group, and total cholesterol, triglyceride, and creatinine were significantly higher in the control NAR group than in the control SDR group. In SDRs, plasma total protein and albumin were significantly lower in the PAN-SDR group than in the control SDR group, and total cholesterol, triglyceride, and creatinine were significantly higher in the PAN-SDR group than in the control SDR group. In NARs, plasma total protein and albumin were not significantly different between the control NAR group and PAN-NAR group. Plasma total cholesterol, triglyceride, and creatinine were significantly higher in the PAN-NAR group than in the control NAR group, but the PAN-induced change of plasma creatinine was less in NARs than in SDRs.

Glomerular damage in nephrotic rats. Electron microscopy experiments were performed to determine the structural changes in glomelular morphology (Fig. 2*A*). The glomeruli in the control SDR and control NAR groups had tall and slender foot processes without foot process effacement (Fig. 2*A*,*a* and *c*). Compared with the control groups (Fig. 2*A*,*a* and *c*), the glomeruli in the PAN-SDR and PAN-NAR groups showed complete foot process effacement with increased dense materials (Fig. 2*A*,*b* and *d*). Lysosomes in the podocytes of the PAN-SDR group were prominent (Fig. 2*A*,*b*), whereas those of the PAN-NAR group were indistictive (Fig. 2*A*,*d*). To confirm PAN-induced glomerular podocyte damage, levels of nephrin protein, a molecule of the slit diaphragm of the podocyte, which is known to be reduced by PAN treatment (14), and levels of dendrin protein, a novel component of the slit diaphragm with proapoptotic signaling properties (2), which is also known to be reduced by PAN treatment (5), were examined in isolated glomeruli of the four groups. Levels of nephrin protein were not significantly different between the control SDR group and control NAR group. Compared with the corresponding control group, levels of nephrin protein were significantly decreased to 46% and 38% in the PAN-SDR group and PAN-NAR group, respectively, and were not significantly different between the PAN-SDR group and PAN-NAR group (Fig. 2*B*). Levels of dendrin protein were not significantly different between the control SDR group and control NAR group. Compared with the corresponding control group, levels of dendrin protein were significantly decreased to 72% and 68% in the PAN-SDR group and PAN-NAR group, respectively, and were not significantly different between the PAN-SDR group and PAN-NAR group (Fig. 2*C*).

Table 1. *Effect of PAN on plasma parameters in SDRs and NARs*

	SDRs		NARs	
	Control	PAN	Control	PAN
Total protein, g/dl	6.1 ± 0.4	5.7 ± 1.3*	5.5 ± 0.4†	5.5 ± 0.3
Albumin, g/dl	3.7 ± 0.3	2.1 ± 0.3*	1.5 ± 0.2†	1.5 ± 0.1
Total cholesterol, mg/dl	65.7 ± 17.1	203.5 ± 64.9*	182.3 ± 25.9†	436.6 ± 75.3*
Triglyceride, mg/dl	40.1 ± 24.1	86.3 ± 25.6*	61.6 ± 16.9†	129.7 ± 44.3*
Free fatty acid, meq/l	0.76 ± 0.29	0.69 ± 0.15	0.69 ± 0.40	0.70 ± 0.39
Urea nitrogen, mg/dl	16.0 ± 2.2	14.1 ± 1.9	14.2 ± 1.7	13.3 ± 0.8
Creatinine, mg/dl	0.13 ± 0.07	0.97 ± 0.66*	0.19 ± 0.09†	0.29 ± 0.10*

Values are expressed as means ± SE; $n = 6$ rats/group. PAN, puromycin aminonucleoside; SDRs, Sprague-Dawley rats; NARs, Nagase analbuminemic rats. *$P < 0.05$ compared with the corresponding control group; †$P < 0.05$ compared between the control groups.

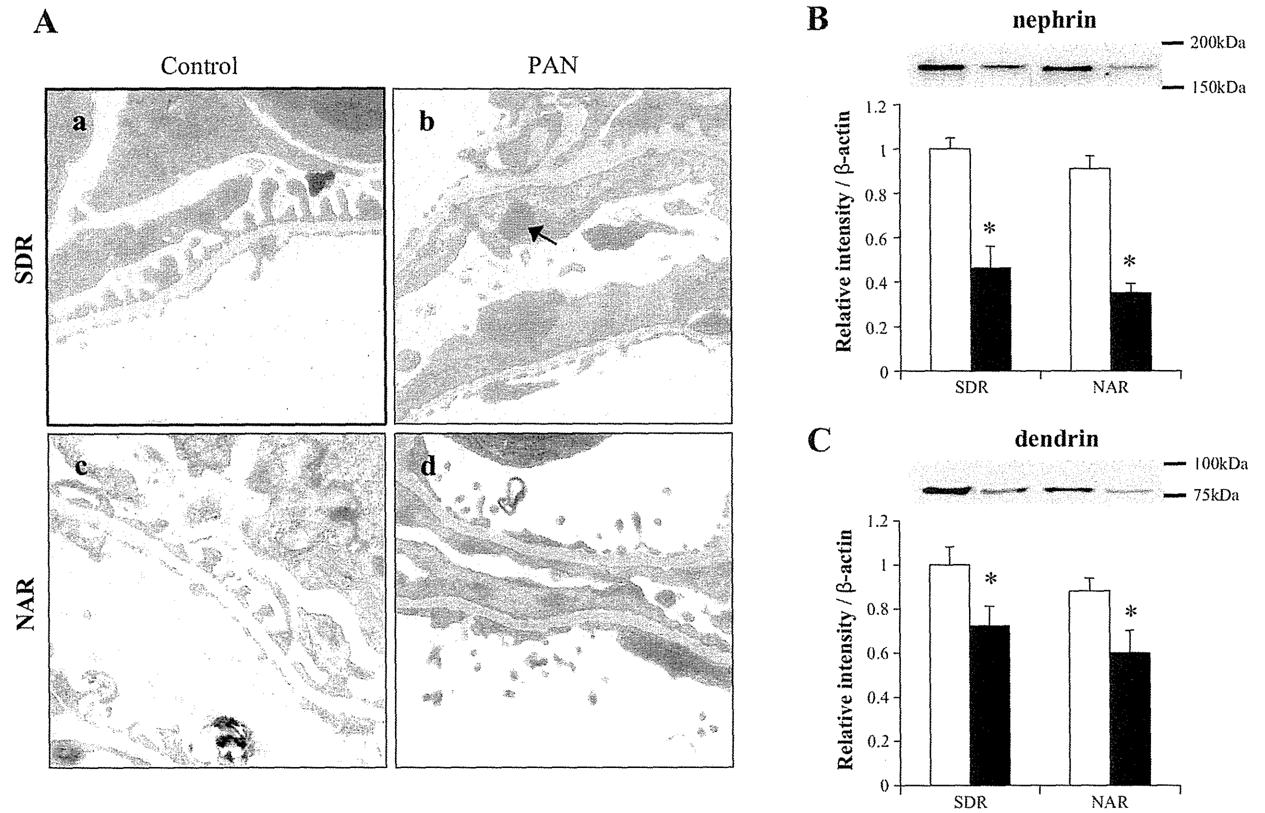

Fig. 2. Effects of PAN treatment on glomerular damages in SDRs and NARs. *A*: representative photomicrographs of transmission electron micrographs of glomeluli in the control SDR group (*a*), PAN-SDR group (*b*), control NAR group (*c*), and PAN-NAR group (*d*) on the 14th day after PAN treatment (magnification: ×5,000). The glomeruli in the control SDR and control NAR groups had tall and slender foot processes without foot process effacement (*a* and *c*). Compared with control groups (*a* and *c*), the glomeruli in the PAN-SDR and PAN-NAR groups showed complete foot process effacement with increased dense materials (*b* and *d*). Lysosomes in the podocytes of the PAN-SDR group were prominent (arrow, *b*), whereas those of the PAN-NAR group were indistict (*d*). *B* and *C*: protein levels of nephrin (*B*) and dendrin (*C*) in isolated glomeruli on the 14th day after PAN treatment were compared between the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots are shown at the *top*, and each lane from the *left* to the *right* was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for each protein were normalized to those for β-actin (*bottom*), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; $n = 6$ rats/group. $^*P < 0.05$ compared with the corresponding control group.

Tubular damage, apoptosis, and lipid accumulation in nephrotic rats. Urinary NAG, a marker of acute PTEC injury (45), was significantly higher in the control NAR group than in the control SDR group and significantly higher in the PAN-SDR group than in the control SDR group (Fig. 3*A*). Urinary 8-OHdG, a marker of oxidative stress (24), was significantly higher in the control NAR group than in the control SDR group and significantly higher in the PAN-SDR group than in the control SDR group (Fig. 3*B*). In contrast to SDRs, urinary NAG or 8-OHdG was not significantly different between the control NAR group and PAN-NAR group (Fig. 3, *A* and *B*).

Apoptosis in the kidney cortex was examined by Western blot and immunohistochemical analyses for caspase 3, a family of cysteine proteases acting as molecular executioners for programmed cell death (22). Levels of caspase 3 protein were significantly higher in the PAN-SDR group than in the control SDR group but were not significantly different among the control SDR, control NAR, and PAN-NAR groups (Fig. 3*C*). Representative photomicrographs of immunohistochemical analysis for caspase 3 are shown in Fig. 3*D*. Strong stainings for caspase 3 protein were detected in several PTECs and distal tubular cells in the kidney cortex of the PAN-SDR group (Fig. 3*D*,*b*), but staining was barely detected in the kidney cortex of the control SDR, control NAR, and PAN-NAR groups (Fig. 3*D*,*a*, *c*, and *d*).

To clarify lipid accumulation, kidney sections were stained with oil red O, and representative photomicrographs are shown in Fig. 4*A*. Oil red O stain was barely detected in several PTECs of the control SDR group and control NAR group (Fig. 4*A*,*a* and *c*). Oil red O stain identified noteworthy amounts of lipoid deposits in the kidneys of the PAN-SDR group (Fig. 4*A*,*b*), and little lipid stained in the kidneys of the PAN-NAR group (Fig. 4*A*,*d*). Free FA content and triglyceride content in the kidney cortex were significantly higher in the PAN-SDR group than in the control SDR group (Fig. 4*B* and *C*). In contrast to SDRs, free FA content and triglyceride content in the kidney cortex were not significantly different between the control NAR group and PAN-NAR group (Fig. 4, *B* and *C*).

Expressions of enzymes for FA metabolism in the kidneys of SDRs and NARs. To clarify the mechanism of lipid accumulation in the kidneys of the PAN-SDR group, expressions of enzymes for FA metabolism in the kidney cortex were com-

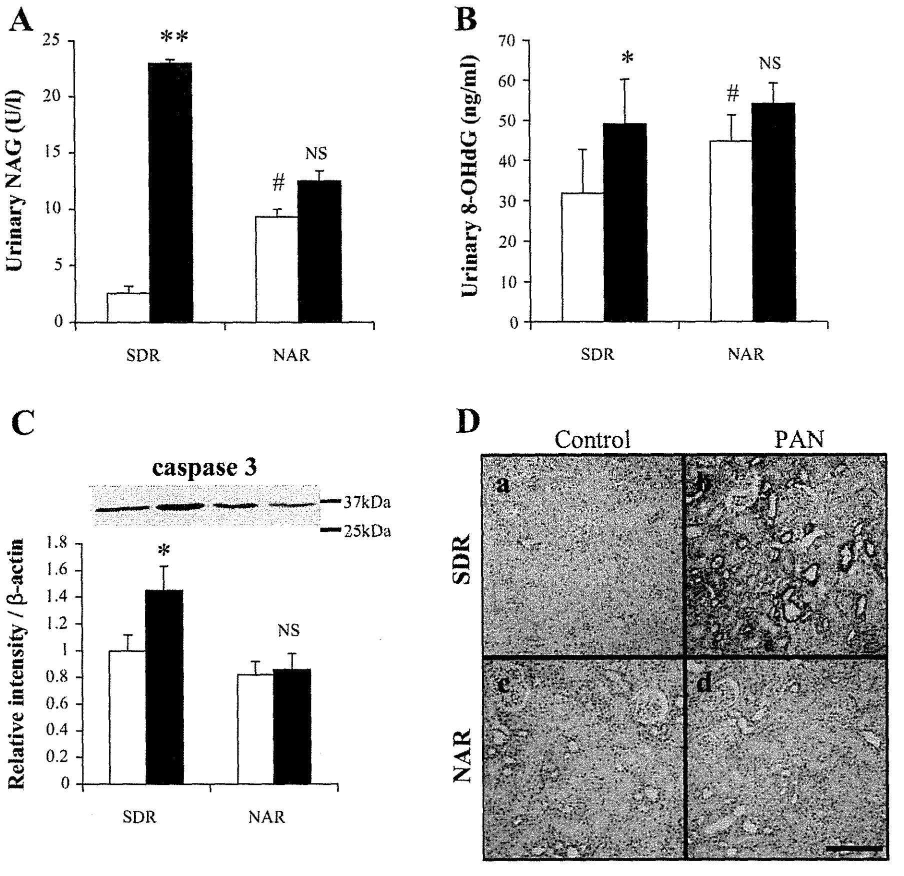

Fig. 3. Effects of PAN treatment on tubular damage in SDRs and NARs. Urinary *N*-acetyl-β-D-glucosaminidase (NAG; *A*) and 8-hydroxydeoxyguanosine (8-OHdG; *B*) and levels of caspase-3 protein in the kidney cortex (*C*) on the 14th day after PAN treatment were compared with the control group (open bars) and PAN-treated group (closed bars) of either SDRs or NARs. Representative Western blots are shown at the *top*, and each lane from the *left* to the *right* was loaded with a protein sample prepared from the control SDR group, PAN-SDR group, control NAR group, and PAN-NAR group. Intensities of the bands for caspase 3 were normalized to those for β-actin (*bottom*), and the intensity of the band in the control SDR group was assigned a value of 1. Data are presented as means ± SE; $n = 6$ rats/group. $*P < 0.05$ and $**P < 0.01$ compared with the corresponding control group; $\#P < 0.05$ between the control groups. *D*: representative photomicrographs of the immunostained kidney cortex for caspase 3 in the control SDR group (*a*), PAN-SDR group (*b*), control NAR group (*c*), and PAN-NAR group (*d*) (magnification: ×200). Bar = 100 μm.

pared among the four groups by Western blot analysis. Levels of the enzymes for FA synthesis (FAS and ACC) were not significantly different among the four groups (Fig. 5, *A* and *B*). Levels of the enzymes for FA transport (L-FABP and CPT-I) were not significantly different among the four groups (Fig. 5, *C* and *D*).

Levels of the enzymes for FA oxidation (VLCAD, LCAD, MCAD, SCAD, ACOX, and CYP4A) were not significantly different between the control SDR group and control NAR group (Fig. 6). Compared with the control SDR group, levels of MCAD protein were significantly decreased to 35% in the PAN-SDR group but were not significantly different between the control NAR group and PAN-NAR group (Fig. 6*C*). Compared with the corresponding control group, levels of CYP4A protein were significantly decreased to 49% and 68% in the PAN-SDR group and PAN-NAR group, respectively (Fig. 6*F*). Levels of the other enzymes for FA oxidation (VLCAD, LCAD, SCAD, and ACOX) were not significantly different between the control group and the corresponding PAN-treated group (Fig. 6, *A*, *B*, *D*, and *E*).

Levels of the transcriptional regulators for MCAD and CYP4A (PPAR-α, PGC-1α, and ERRα) were not significantly different between the control SDR group and control NAR group (Fig. 7). Levels of PPAR-α protein were not significantly different between the control group and the corresponding PAN-treated group (Fig. 7*A*). Two distinct bands (~115 and ~130 kDa) for PGC-1α protein were detected by Western blot analysis (Fig. 7*B*). Three isoforms of PGC-1α (PGC-1α-a, PGC-1α-b, and PGC-1α-c) have been cloned from mouse skeletal muscles, but PGC-1α-a is only isoform expressed in the mouse liver (22). The PGC-1α isoforms that are expressed in the kidney are unknown; however, the protein size of the lower band for PGC-1α protein in rat kidney samples is identical with that of a single band for PGC-1α protein in rat liver samples (date not shown). Compared with the control SDR group, levels of the lower band for PGC-1α protein were significantly decreased to 37% in the PAN-SDR group but were not significantly different between the control NAR and PAN-NAR groups (Fig. 7*B*). Levels of the upper band for PGC-1α protein were not significantly different between the control group and the corresponding PAN-treated group. Compared with the control SDR group, levels of ERRα protein were significantly decreased to 31% in the PAN-SDR group but were not significantly different between the control NAR and PAN-NAR groups (Fig. 7*C*).

Expressions of MCAD, CYP4A, PGC-1α, and ERRα proteins were further examined by immunohistochemistry in the kidney cortex of the control SDR and PAN-SDR groups. The representative photomicrographs are shown in Fig. 8. Strong stainings for MCAD, CYP4A, PGC-1α, and ERRα proteins were detected in PTECs of the control SDR group, and weak stainings for these proteins were detected in the distal tubules, glomeruli, and renal vessels (Fig. 8, *a–d*). Immunohistochemical experiments confirmed marked decreases in the levels of MCAD, CYP4A, PGC-1α, and ERRα proteins in PTECs of the PAN-SDR group (Fig. 8, *e–h*).