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ARTICLE

The relationship between skin ultrasound images and muscle damage using skin blotting in wheelchair basketball athletes

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Abstract

Study design Secondary analysis of a cross-sectional observation study.

Objectives To determine the relationship between skin ultrasound images and muscle damage in wheelchair basketball athletes, using skin blotting examinations of the ischial regions.

Setting Community, Japan.

Methods Fourteen elite wheelchair basketball athletes were recruited. We obtained data regarding participants' characteristics. We undertook ultrasonographic images and quantitative skin blotting of the ischial region before and after training, and after rest.

Results We identified Category II and III pressure injuries in 2 of the 12 participants. Structural features were classified into four categories based on ultrasonographic features, namely, normal skin structure, unclear superficial and deep fascia, cloudy fat layer, and fat infiltration and low-echoic lesion/anechoic lesions. The muscle-type creatinine kinase (CK-M) level (median [interquartile range: IQR], 2.98 [2.80–3.47]) in the fat infiltration and low-echoic lesion/anechoic lesion group was significantly higher (1.43 [1.41–1.49]) than in a nonfat infiltration and low-echoic lesion/anechoic lesion group after training ($p = 0.03$). The interleukin-6 (IL-6) level (median [IQR], 23.5 [16.15–58.97]) in the fat infiltration and low-echoic lesion/anechoic lesion group was significantly higher (1.94 [1.74–4.44]) than in the nonfat infiltration and low-echoic lesion/anechoic lesion group after rest (mean difference = −25.4, 95% CI −61.1 to 10.7, $p = 0.03$).

Conclusions The combination of ultrasonographic images and skin blotting using CK-M and IL-6, could detect early deep tissue damage in wheelchair athletes. These techniques could be potentially useful in the treatment and prevention of pressure injuries.

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Introduction

Wheelchair basketball was included as a competitive sport at the inaugural Rome 1960 Paralympic Games, and is a popular sport for people with disabilities. Wheelchair basketball can be played by people with physical disabilities that involve reduced function of the lower limb or paralysis of the lower portion of the body due to several causes, including spinal cord injury (SCI). Participation in sport, including wheelchair basketball, has been reported to improve psychological status, quality of life, and community integration; [1, 2] however, wheelchair players risk secondary disabilities such as pressure injuries (PIs) [3, 4].

A PI is a localized injury to the skin and/or underlying tissue, usually over a bony prominence, as a result of pressure, or pressure in combination with shear stress [5]. Risk factors in the development of a PI have previously

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been reported, and include activity limitation, mobility limitation, increased friction and shear stresses, poor nutritional status, skin moisture issues, increased body temperature, an advanced age, a loss of sensory perception, or SCI [5]. Wheelchair basketball athletes have additional risks due to the often difficult and complicated physical performance, thus increasing the risk of developing PIs. The effects of PIs for wheelchair basketball athletes include pain, discomfort, and infection [6, 7], and can also include poor performance and an inability to participate in play and retain their team position due to rehospitalization [8, 9]. Therefore, PI prevention for wheelchair basketball athletes is important.

The prevalence rates for PIs among wheelchair basketball athletes have been reported to be between 27 and 68% [10–12], whereas the prevalence of PIs in people with SCI after 5 years has been reported to be 13% [13]. The risk of PI development in wheelchair basketball athletes is higher than that of their nonathletic counterparts. The high prevalence of PIs, including deep tissue injury (DTI), in wheelchair basketball athletes may be attributable to undue pressure or friction/shear stresses due to 'stop and dash' requirement of the sport, or to the heavy crashes that occur during competition. Previous studies using ultrasonography reported prevalence rates of 45% and 68% for DTI in elite male and female wheelchair basketball athletes, respectively [11, 12]. These studies were complete surveys conducted in the male and female national teams, and images of the sacral and ischial regions were analyzed. Other investigators have reported that PI occurrence via visual inspection was 2%; however, in another study using ultrasonography, low-echoic lesions were identified as DTI in 13% of study participants with SCI [14]. A suspected DTI is defined as "intact or nonintact skin with a localized area of persistent non-blanchable deep red, maroon, or purple discoloration, or epidermal separation revealing a dark wound bed or blood-filled blister. Pain and temperature change often precede skin color changes" [15]. However, it is not sufficient to detect suspected DTI through an assessment of skin appearance. In a clinical setting, ultrasonography can be used to detect suspected DTI, and this has been especially useful in observing unclear layered structures, heterogeneous hypoechoic lesions, and discontinuous fascia within the subcutaneous fat tissue layer [16]. One study showed tissue changes such as muscle atrophy or fat infiltration into the muscles of people with SCI [17]. However, using ultrasonography alone, it is difficult to detect whether skin tissue including adipose and muscle tissue in a DTI area has actually sustained damage. Early detection of deep tissue damage including damage to muscle tissue can likely prevent PIs.

One simple and noninvasive method that can be used instead of a histological biopsy examination is the skin blotting method [18]. With this method, a negatively charged nitrocellulose membrane is hydrated with normal saline, and is attached to the skin surface for 10 min. The membrane attracts soluble protein from the inside of the attached skin tissue. Immunostaining of the blotted membrane reflects the pathophysiological status of the skin [19, 20]. Minematsu et al. [18] demonstrated the detection of fluorescein-labeled dextran released from implanted agarose gel in the dorsal skin of mice. Their results showed that fluorescein-labeled dextran from the subcutaneous adipose tissue could be detected 1 h following attachment of the membrane to the skin surface [18]. Evaluating the condition of local muscle using the skin blotting method has previously been undertaken [21]. Therefore, we used this method to identify deep tissue damage, including muscle tissue in the ischial region, in wheelchair basketball athletes.

This study aimed to determine the relationship between skin ultrasound images and muscle damage using a skin blotting examination of the ischial region in wheelchair basketball athletes. We confirmed the relationship between skin ultrasound images and muscle damage using a skin blotting examination before and after training, and after rest.

Methods

Research design

We undertook a secondary analysis of a cross-sectional observation study. The survey was conducted during a training camp for elite Japanese male wheelchair basketball athletes in November 2017.

Participants

The eligibility criterion was elite Japanese male wheelchair basketball athletes, who participated in a training camp in November 2017. Participants whom team staff considered to have difficulties in joining the study, were excluded.

Study protocol

Researchers performed physical examinations and interviewed participants. A wound, ostomy, and continence nurse (WOCN) visually inspected and palpated the skin of the ischial regions. A sonographer took ultrasonographic images of the bilateral ischial tuberosities using ultrasonographic diagnostic equipment. Finally, the WOCN and researchers collected skin blotting samples from the unilateral ischial region, where tissue damage was assessed by the WOCN to be more severe than the contralateral ischial region, in the morning (baseline) and at night (after training) on the first day, and on the following morning (after rest).

Measurements

Participant characteristics

Participant characteristics were obtained through interviews, involving participants' age, height, weight, cause of disability, duration of disease, assistive devices required for daily life, the cushion type for daily activities/basketball wheelchair use, wheelchair basketball participation history, past history of a pressure injury, and any surgical history of flap reconstruction for a pressure injury. One researcher measured the triceps skinfold thickness (TSF) and the arm muscle circumference (AMC) on the nondominant upper arm of the athletes three times using a skinfold caliper (Abbott Japan, Tokyo, Japan) and a measuring tape (Abbott Japan, Tokyo, Japan), respectively, and the average was calculated.

Skin structure changes

A sonographer took B-mode ultrasonographic images of bilateral ischial tuberosities with ultrasonographic diagnostic equipment using a linear-array (5–18 MHz) transducer (Noblus; Hitachi Aloka Medical, Ltd., Tokyo, Japan). Gain and tissue depth for later quantitative image analysis were maintained at constant levels. The athletes were placed in a lateral position and a sonographer measured their skin/fat layer and muscle layer. Finally, the sonographer, the WOCN, and the researchers performed qualitative classification as per the morphological texture of the skin structure changes. The categories were generated by identifying similar morphological textures, and were named accordingly. In addition, ultrasonographic images of beef muscle (beef rump and marbled beef muscle) samples were obtained by the sonographer using the same ultrasonographic diagnostic equipment for comparison with the players' images.

Properties of histological deep tissue damage measured using skin blotting (CK-M, IL-6)

A piece of nitrocellulose membrane (Bio-Rad, Hercules, CA) was attached to the sampling site following hydration with normal saline. A quantitative skin blotting examination was performed to measure the muscle damage and the inflammatory response at the ischial region in the participants [18, 19]. We selected muscle-type creatinine kinase (CK-M) and Interleukin 6 (IL-6) as markers of muscle damage. CK-M is related to muscle disruption, and peaks between 24 h and 72 h after physical training [22]. IL-6 is secreted from muscle tissue in response to an inflammatory response [23].

A piece of nitrocellulose membrane (Bio-Rad, Hercules, CA) was moistened with 50 uL of normal saline, and then attached to the skin surface of the selected ischial region for 10 min with players lying in a prone position. The collected samples were dried and stored at 4 °C until staining. The staining and evaluating processes for CK-M and IL-6 were blinded. The immunostaining procedure, using a vacuum-driven immunodetection system (SNAP i.d. 2.0, Merck Millipore, Billerica, MA), was as follows. The blotting samples were divided into two pieces and blocked with blocking solution (Blocking One, Nacalai Tesque, Kyoto, Japan) for 10 min. The target proteins were detected through a 10 min reaction time with primary antibodies, namely, the rabbit polyclonal anti-CK-M antibody (dilution 1:300; Proteintech Group, Inc., Rosemont, IL), and the rabbit monoclonal anti-IL-6 antibody (dilution 1:500; Cell Signaling Technology, Danvers, MA), followed by detection through a 10 min reaction time with horseradish peroxidase conjugated donkey anti-rabbit Ig (dilution 1:1000; Jackson ImmunoResearch Laboratories, West Grove, PA). The dot blot samples of the appropriate recombinant peptides, that is, human recombinant CK-M (Proteintech, Rosemont, IL) and human recombinant IL-6 (Cell Signaling Technology) and their solvents, were prepared and simultaneously stained as positive and negative controls. Immunoreactivities were visualized with chemiluminescent substrates for peroxidase (Luminata Forte, Merck Millipore), and recorded using a chemiluminescence imaging device (LumiCube Liponics, Tokyo, Japan). The immunostaining images were analyzed according to threshold values, which comprised the mean $+2\times$ the standard deviation of the negative control samples, and we calculated the percentage of the positive area to the total area of samples using Image J (National Institutes of Health, Bethesda, MD). The CK-M value was determined as the relative value divided by the CK-M value obtained before training. The IL-6 value was used as the absolute value.

Analysis

Descriptive statistics for characteristics were calculated as the median (interquartile range: IQR). Structural features of ultrasonographic images were grouped according to the presence or absence of low-echoic/anechoic lesions. A Mann–Whitney U test was used to compare outcomes between the two groups. A *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS®24.0 for Windows® (SPSS Inc., Chicago, IL, USA).

Results

Fourteen male elite wheelchair basketball athletes were recruited. Two athletes were excluded because of significant edema identified in the ischial region and a lack of

Table 1 Characteristics of participants.

			$N = 12$	
Age (years)			27	(23.5–31.0)
Height (cm)			172.8	(164.3–176.3)
Weight (kg)			63.2	(56.8–68.8)
Triceps skinfold thickness (mm)			8.3	(6.0–13.7)
Arm muscle circumference (cm)			35.5	(33.2–36.5)
Cause of disability	Spinal cord injury		8	(66.7)
	Spinal tumor		1	(8.3)
	Spinal Bifida		1	(8.3)
	Lower limb amputation		2	(16.7)
Duration of disease (years)			14.5	(10.0–18.3)
Sensory disturbance			10	(83.3)
Assistive device for daily life		Wheelchair	12	(100.0)
		Prosthetic foot	2	(16.7)
Cushion	Daily wheelchair use	Air cell	7	(58.3)
		Gel	1	(8.3)
		Urethane foam	4	(33.3)
	Basketball wheelchair use	Urethane foam	12	(100.0)
Wheelchair basketball (years spent playing)			8.0	(6.0–11.3)
History of pressure injuries			10	(83.3)
Flap reconstruction for pressure injury			3	(25.0)

Median (interquartile range)/N (%).

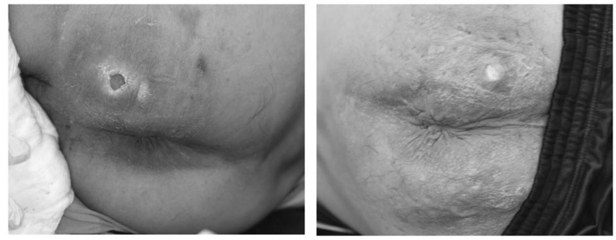


Fig. 1 Cases with pressure injuries (PIs). One athlete had a Category II PI on the left ischial region and another athlete had a Category III PI on the right ischial region.

ultrasonographic images due to the excessive thickness for linear-array transducers, and twelve participants were analyzed. Table 1 shows the participants' characteristics. The median age was 27 years (IQR, 23.5–31.0), the TSF and the AMC were 8.3 (IQR, 6.0–13.7) mm and 35.5 (IQR, 33.2–36.5) cm, respectively, and ten athletes were identified as having sensory disturbance. All athletes used wheelchairs as assistive devices for daily life, and used urethane foam cushions when in their basketball wheelchairs. Ten athletes with sensory disturbance reported a past history of PIs. At the time of this study, Category II and III PIs on the ischial region were identified in two participants on visual inspection (Fig. 1).

A total of 24 ultrasonographic images, obtained from the bilateral ischial region in 12 participants, were collected for qualitative analysis. Skin structural features were classified into four categories, namely, normal skin structure (three images), unclear superficial and deep fascia (nine images), cloudy fat layer (six images), and fat infiltration and low-echoic lesion/anechoic lesion (six images) (Fig. 2). The normal skin structure category showed skin structures that clearly distinguished the dermis/epidermis, the subcutaneous tissue, and the muscle layer. The unclear superficial and deep fascia category identified superficial fascia in the subcutaneous tissue and deep fascia in the muscle layer that were discontinuous or unclear. A cloudy fat layer categorization indicated unclear superficial fascia, with low contrast and rough resolution. A fat infiltration and low-echoic lesion/anechoic lesion categorization revealed a low-echoic/anechoic lesion with fat infiltration into the muscle. This latter finding confirmed deep tissue damage.

We took ultrasonographic images of beef rump and marbled beef muscle samples, to understand the skin structure changes in wheelchair basketball athletes. In the ultrasonographic images of beef rump muscle, the superficial fascia was clear. However, in the ultrasonographic images of marbled beef muscle, the structures of the layers were unclear and of low contrast (Fig. 3).

In the skin blotting results, the CK-M levels in all 12 participants decreased from after training to after rest. In one participant, the CK-M level (3.5%) maintained a similar level until after rest (3.2%). The IL-6 level varied after training and at rest according to each individual (Fig. 4).

We analyzed the relationship between skin ultrasonography images and muscle damage using skin blotting. The features classified according to the qualitative method were divided into two groups, depending on the presence or absence of deep tissue damage, namely, a fat infiltration and low-echoic lesion/anechoic lesion group, and a nonfat infiltration and low-echoic lesion/anechoic lesion group. The CK-M level (median [IQR]), 2.98 [2.80–3.47]) in the fat infiltration and low-echoic lesion/anechoic lesion group was significantly higher (1.43 [1.41–1.49]) than that of the nonfat infiltration and low-echoic lesion/anechoic lesion group after training ($Z = -2.19$, $p = 0.03$) (Table 2). No significant difference was found in the CK-M level after rest or in the IL-6 level after training, ($Z = -1.38$, $p = 0.17$; $Z = -1.38$, $p = 0.17$, respectively). The level of IL-6 (median [IQR], 23.5 [16.2–59.0]) in the fat infiltration and low-echoic lesion/anechoic lesion group was significantly higher (1.94 [1.74–4.44]) than that of the nonfat infiltration and low-echoic lesion/anechoic lesion group after rest ($Z = -2.19$, $p = 0.03$) (Table 2).

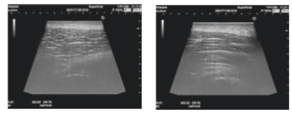
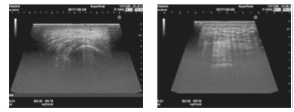
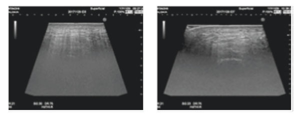
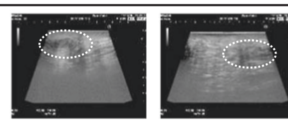
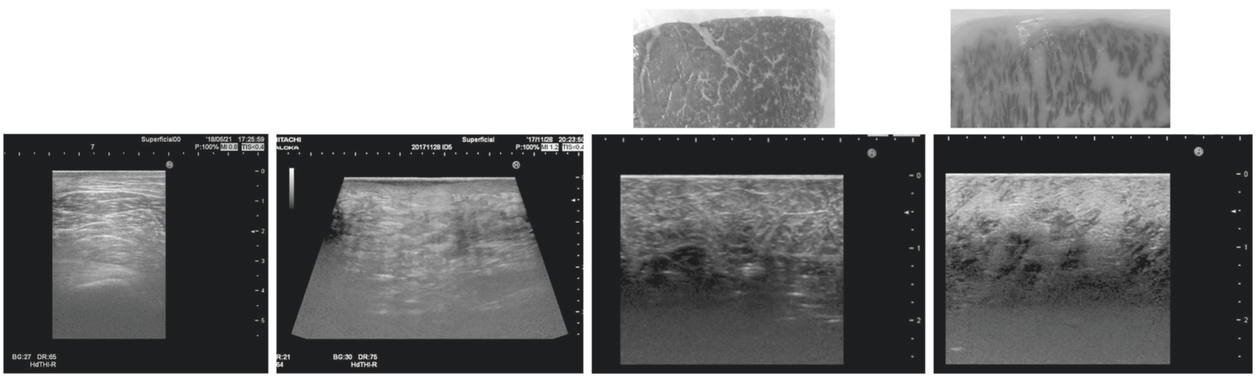
	Typical Ultrasonographic images	Number of images
Normal skin structure		3
Unclear superficial and deep facia		9
Cloudy fat layer		6
Fat infiltration and low-echoic lesion/anechoic lesion		6

Fig. 2 Classification of tissue changes in the ischial region using ultrasonography.



a. Able-bodied individual b. Athlete with spinal cord injury c. Beef rump muscle d. Marbled beef muscle

Fig. 3 Ultrasonographic images. a The skin layer structure in ultrasonographic image of an able-bodied individual is clear. **b** The muscle layer is not able to be distinguished from the fat layer in this ultrasonographic image of an athlete with SCI. **c** This ultrasonographic image of beef rump muscle shows clear superficial fascia. **d** This ultrasonographic image of marbled beef muscle shows fat infiltration.

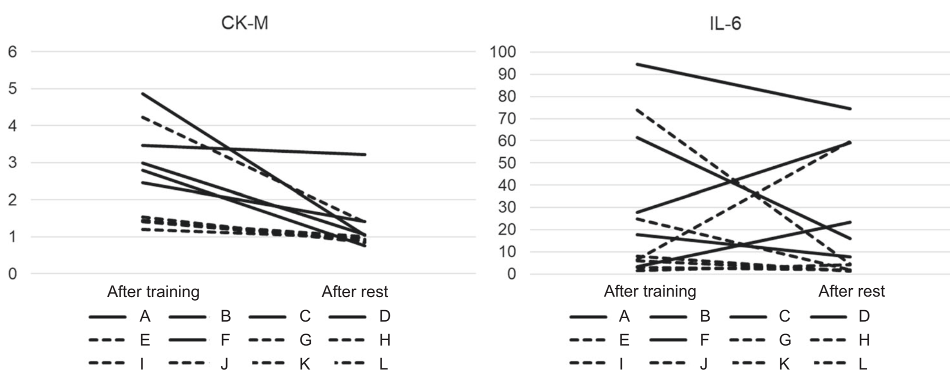


Fig. 4 Changes in tissue damage using skin blotting. CK-M and IL-6 were calculated as the proportion of positive area (PPA) ($N = 12$). PPA = (pixels of positive area for each protein)/(pixels of analyzed area of nitrocellulose membrane). The CK-M value was used as the relative value divided by the CK-M value obtained before training. A–D, F Solid lines show the changes in values in the athletes with ultrasonographic images categorized as fat infiltration and low-echoic lesion/anechoic lesion. E, G–L Dotted lines show the changes in values in the athletes without ultrasonographic images categorized as fat infiltration and low-echoic lesion/anechoic lesion.

Table 2 The relationship between the pattern of fat infiltration and low-echoic lesion/anechoic lesion and tissue damage. ($N = 12$).

	Ultrasonographic image of fat infiltration and low-echoic lesion/anechoic lesion		Z	P
	Yes ($n = 5$)	No ($n = 7$)		
CK-M after training	2.98 (2.80–3.47)	1.43 (1.41–1.49)	−2.19	0.03
CK-M after rest	1.06 (1.05–1.41)	0.94 (0.89–0.99)	−1.38	0.17
IL-6 after training	27.7 (17.68–61.33)	6.61 (4.45–16.36)	−1.38	0.17
IL-6 after rest	23.5 (16.15–58.97)	1.94 (1.74–4.44)	−2.19	0.03

Median (interquartile range).

The CK-M value was used as the relative value divided by the CK-M value obtained before training.

The value of IL-6 was used as the absolute value.

Discussion

This is the first study to investigate the relationship between skin structure changes and properties of deep tissue damage, involving muscle tissue of the ischial region in elite Japanese male wheelchair basketball athletes. The level of CK-M after training and the level of IL-6 after rest in the fat infiltration and low-echoic lesion/anechoic lesion group showed significantly higher values than those in the nonfat infiltration and low-echoic lesion/anechoic lesion group after training.

Six of 24 images (25%) of the ischial region showed features of deep tissue damage among the participants. The findings concerning the fat infiltration and low-echoic lesion/anechoic lesion group in this study were similar to DTI findings in a previous study using ultrasonography [24]. The fat infiltration and low-echoic lesion/anechoic lesion group showed the following findings: an unclear layered structure, a hypoechoic lesion, discontinuous fascia, and a heterogeneous hypoechoic area. In our study, four ischial regions, excluding two ischial regions with PIs, showed no suspected DTI. The National Pressure Ulcer Advisory Panel [25] has defined DTI as "intact or nonintact skin with a localized area of persistent non-blanchable deep red, maroon, purple discoloration, or epidermal separation revealing a dark wound bed or blood-filled blister" (p. 594). Kanno et al. reported that a normal area on inspection and palpation showed a low-echoic lesion [14]. Therefore, ultrasound examinations should be performed early to detect the deep tissue damage for wheelchair basketball athletes.

The findings concerning fat infiltration detected in this study appeared similar to that of the marbled beef muscle sample, in which superficial fascia was unclear, and the area of subcutaneous and muscle tissues showed high intensity and tissue deformation. A previous study investigating anatomical features reported that the amount of muscle tissue in individuals with SCI was less than that of healthy people [26]. All the participants with fat infiltration and low-echoic lesion/anechoic lesions in this study had SCI. However, ultrasonographic images in participants with lower limb amputation showed clear layer structures. Therefore, the ultrasonographic features in this study were attributable to the deformation and invasion of adipose tissue into muscle tissue, with accompanying muscle atrophy, related to dysfunction caused by lower body paralysis. These structural changes may reduce tissue tolerance to external pressures in wheelchair basketball athletes with SCI.

The level of CK-M after training and the level of IL-6 after rest using skin blotting in the fat infiltration and low-echoic lesion/anechoic lesion group, were significantly higher than in the nonfat infiltration and low-echoic lesion/anechoic lesion group after training. These findings supported our hypothesis. As mentioned previously, the fat infiltration and low-echoic lesion/anechoic lesion group showed evidence of discontinuous fascia and skin deformation that included a mixture of subcutaneous tissue and muscle. A previous study has reported that there was a significant association between gluteal muscle damage with subcutaneous edema and inflammation, and serum CK levels [27]. Hence, CK-M level markers obtained after training, and IL-6 level markers obtained after rest on the ischial region using skin blotting may indicate muscle damage. In the clinical setting, an assessment in combination with the CK-M and IL-6 level tests could identify muscle damage that requires an adequate rest time, and an appropriate selection of daily activity/basketball wheelchair cushions for controlling pressure, or vibration therapy to promote the blood supply [28]. Therefore, we propose a combined examination of ultrasonographic images and skin blotting using CK-M and IL-6 markers for early detection of deep tissue damage in wheelchair athletes.

The scope of this study was limited by its small sample size, which may have decreased its statistical power. It is also possible that the sample was not reflective of all wheelchair athletes. Despite these limitations, the measurement error was reduced by performing ultrasonography, skin blotting, and staining of the skin blotting membrane by individual specialists.

Conclusion

The levels of CK-M after training and IL-6 after rest using skin blotting in a fat infiltration and low-echoic lesion/anechoic lesion group were significantly higher than in a nonfat infiltration and low-echoic lesion/anechoic lesion group. A combination of ultrasonographic images and skin blotting using CK-M and IL-6 markers could help ensure detection of early deep tissue damage including muscle damage in wheelchair athletes and, therefore, prevent PI progression.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions NT conceived the research question, designed the study, collected the data, analyzed the data, interpreted the data, and wrote the paper. TM, TMA, and KY conceived the design of the study, collected the data, analyzed the data, interpreted the data, and wrote the paper. HS was responsible for study and protocol design, oversaw the data collection process, and consulted on the data analysis protocols. She contributed to the writing of the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics This study was approved by the Research Ethics Committee of the Graduate School of Medicine, the University of Tokyo (#11765-(3)). We certify that all applicable institutional regulations concerning the ethical use of human volunteers were followed during the course of this research. All athletes provided their informed consent to participate in the study. The study interview, measurements, and examinations were conducted in a private space to ensure all participants' rights to privacy.

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References

1. Gioia MC, Cerasa A, Di Lucente L, Brunelli S, Castellano V, Traballesi M. Psychological impact of sports activity in spinal cord injury patients. Scand J Med Sci Sports. 2006;16:412–6.
2. McVeigh SA, Craven BC, Hitzig SL. Influence of sport participation on community integration and quality of life: a comparison between sport participants and non-sport participants with spinal cord injury. J Spinal Cord Med. 2009;32:115–24.
3. Curtis KA, Dillon DA. Survey of wheelchair athletic injuries: common patterns and prevention. Paraplegia. 1985;23:170–5.
4. Klenck C, Gebke K. Practical management: common medical problems in disabled athletes. Clin J Sport Med. 2007;17:55–60.
5. National Pressure Ulcer Advisory Panel, European Pressure Ulcer Advisory Panel and Pan Pacific Pressure Injury Alliance. In: Background: International NPUAP/EPUAP Pressure Ulcer Classification System. Haesler E, editor. Prevention and treatment of pressure ulcers: quick reference guide. Osborne Park, Western Australia: Cambridge Media; 2014.
6. Spilsbury K, Nelson A, Cullum N, Iglesias C, Nixon J, Mason S. Pressure ulcers and their treatment and effects on quality of life: hospital inpatient perspectives. J Adv Nurs. 2007;57:494–504.
7. Livesley NJ, Chow AW. Infected pressure ulcers in elderly individuals. Clin Infect Dis. 2002;35:1390–6.
8. Paker N, Soy D, Kesikta N. Reasons for rehospitalization in patients with spinal cord injury: 5 years'experience. Int J Rehabilit Res. 2001;29:71–76.
9. Cardenas DD, Hoffman JM, Kirshblum S, Mckinley W. Etiology and incidence of rehospitalization after traumatic spinal cord injury: a multicenter analysis. Arch Phys Med Rehabilit. 2004;85:1757–63.
10. Rocco FM, Saito ET. Epidemiology of sportive injuries in basketball wheelchair players. Acta Fisiatr. 2006;13:17–20.
11. Mutsuzaki H, Tachibana K, Shimizu Y, Hotta K, Fukaya T, Karasawa M, et al. Factors associated with deep tissue injury in male wheelchair basketball players of a Japanese national team. Asia-Pac J Sports Med, Arthrosc, Rehabilit Technol. 2014;1:72–76.
12. Shimizu Y, Mutsuzaki H, Tachibana K, Tsunoda K, Hotta K, Fukaya T, et al. A survey of deep tissue injury in elite female wheelchair basketball players. J Back Musculoskelet Rehabilit. 2017;30:427–34.
13. Chen Y, DeVivo MJ, Jackson AB. Pressure ulcer prevalence in people with spinal cord injury: age-period-duration effects. Arch Phys Med Rehabilit. 2005;86:1208–13.
14. Kanno N, Nakamura T, Yamanaka M, Kouda K, Nakamura T, Tajima F. Low-echoic lesions underneath the skin in subjects with spinal-cord injury. Spinal Cord. 2009;47:225–9.
15. National Pressure Ulcer Advisory Panel. NPUAP position statement on staging – 2017 clarifications. National Pressure Ulcer Advisory Panel; 2017. http://www.npuap.org/wp-content/uploads/2012/01/NPUAP-Position-Statement-on-Staging-Jan2017.pdf
16. Yabunaka K, Iizaka S, Nakagami G, Aoi N, Kadono T, Koyanagi H, et al. Can ultrasonographic evaluation of subcutaneous fat predict pressure ulceration? J Wound Care. 2009;18:192–8.
17. Gefen A. Tissue changes in patients following spinal cord injury and implications for wheelchair cushions and tissue loading: a literature review. Ostomy Wound Manag. 2014;60:34–45.
18. Minematsu T, Horii M, Oe M, Sugama J, Mugita Y, Huang L, et al. Skin blotting: a noninvasive technique for evaluating physiological skin status. Adv Ski Wound Care. 2014;27:272–9.
19. Ogai K, Matsumoto M, Aoki M, Minematsu T, Kitamura K, Kobayashi M, et al. Increased level of tumour necrosis factor-alpha (TNF- α) on the skin of Japanese obese males: measured by quantitative skin blotting. Int J Cosmet Sci. 2016;38:462–9.
20. Koyano Y, Nakagami G, Iizaka S, Sugama J, Sanada H. Skin property can predict the development of skin tears among

elderly patients: a prospective cohort study. Int Wound J. 2017;14:691–7.

21. Neya M, Koboyashi K, Maeda T, Agari M. Skin blotting is a noninvasive technique for evaluating local muscle condition. J Australian Strength Cond. 2018;26:64.
22. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull. 2007;81–82:209–30.
23. Sacheck JM, Cannon JG, Hamada K, Vannier E, Blumberg JB, Roubenoff R, et al. Age-related loss of associations between acute exercise-induced IL-6 and oxidative stress. Am J Physiol. 2006;291:340–9.
24. Aoi N, Yoshimura K, Kadono T, Nakagami G, Iizaka S, Higashino T, et al. Ultrasound assessment of deep tissue injury in pressure ulcers: possible prediction of pressure ulcer progression. Plast Reconstr Surg. 2009;124:540–50.
25. Edsberg LE, Black JM, Goldberg M, McNichol L, Moore L, Sieggreen M. Revised national pressure ulcer advisory panel pressure injury staging system. J Wound, Ostomy Cont Nurs. 2016;43:585–97.
26. Jaxon V. Pressure ulcer risk: the effect of anatomical features on interface pressure and tissue deformation in people with spinal cord injury. Undergraduate Thesis, University of Pittsburgh; 2016 (Unpublished).
27. Hattori Y, Ikeuchi T, Kuroda Y, Matsugi K, Minami S, Higuchi T, et al. Postoperative gluteal skin damage associated with latent development of gluteal muscle damage. J Dermatol. 2016;43:547–52.
28. Arashi M, Sugama J, Sanada H. Vibration therapy accelerates healing of stage i pressure ulcers in older adult patients. Adv Skin Wound Care. 2010;23:321–7.