

Establishment of muscle strength measurement system using aged mice

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Objective

In Japan, the aging rate is very high among developed countries. The increase of medical expenses in a super-aged society has become serious. It is important to maintain good quality of life (QOL) throughout life. One of the factors that lowers QOL of the elderly is a decline in mobility accompanied by a decrease in muscle strength such as sarcopenia. Many food and drug suppliers are developing products to prevent muscle weakness.

In this study, we tried to establish a test system to evaluate effects of therapeutic agents on muscle weakness using aged mice. First, we compared the muscle strength of 13, 41, 54, 80, 93, and 106-week-old C57BL/6J male mice using the whole animal muscle test system (1300A, Aurora Scientific). Compared with 13-week mice, aged mice (54 to 106 weeks) showed significantly lower muscle strength. We also measured Nicotinamide adenine dinucleotide (NAD) concentration in skeletal muscle, which is critical for maintaining the health of our cells, tissues, and bodies.

Moreover, we tested the effects of nicotinamide mononucleotide (NMN, a precursor of NAD), which is recently attracted attention as a dietary supplement with potential anti-aging effects. Oral administration of NMN for 4 weeks showed preventive effect on muscle weakness in aged mice.

The present test system would be useful to evaluate potential efficacy of newly developed therapeutic agents on muscle weakness in the elderly.

Summary in Japanese

日本は先進諸国の中において、高齢化率が非常に高い水準となっており、超高齢化社会における医療費増大の解消が課題となっている。そのため要介護となる状態に至る前に、健康な状態を長く維持し、生涯にわたり高いクオリティ・オブ・ライフ(QOL)を保てることが課題となる。

高齢者のQOLを下げる要因の一つとして、サルコペニアをはじめとする筋力の低下を伴う運動能力の低下が挙げられる。筋力低下の分野については、食品から医薬品まで幅広いメーカーが商品開発をテーマに掲げている。

そこで本研究では、高週齢マウスを用いて、筋力低下に対するサプリメント・医薬品の効果を評価可能な試験系の構築を目的とした。まず、13, 41, 54, 80, 93, 106週齢のC57BL/6Jマウスを使用し、小動物筋力測定装置(Aurora Scientific)を用いた筋力測定を検討することで、筋力低下に対する薬物作用を評価するために最適な週齢を決定した。高週齢マウスの筋力は、13週齢のマウスに比べて著しく低い値を示した。また、老化に深く関わるエネルギー代謝とサーチュイン遺伝子の活性化をつかさどるニコチンアミドアデニンジヌクレオチド(NAD)の骨格筋中濃度を測定した。その結果、106週齢のマウスにおいて骨格筋中のNAD濃度は低い値を示した。

薬効薬理試験として、近年、抗老化作用を示すことが期待されるサプリメントである、ニコチンアミドモノヌクレオチド(NMN, NADの前駆体)を高週齢マウスに4週間経口投与した。筋力測定は、投与開始後2および4週目に行った。本試験条件下において、NMNの投与により高週齢マウスの筋力低下を抑制することが示唆された。

Materials and Methods

Experiment 1: Muscle strength and NAD in aged mice

Animals

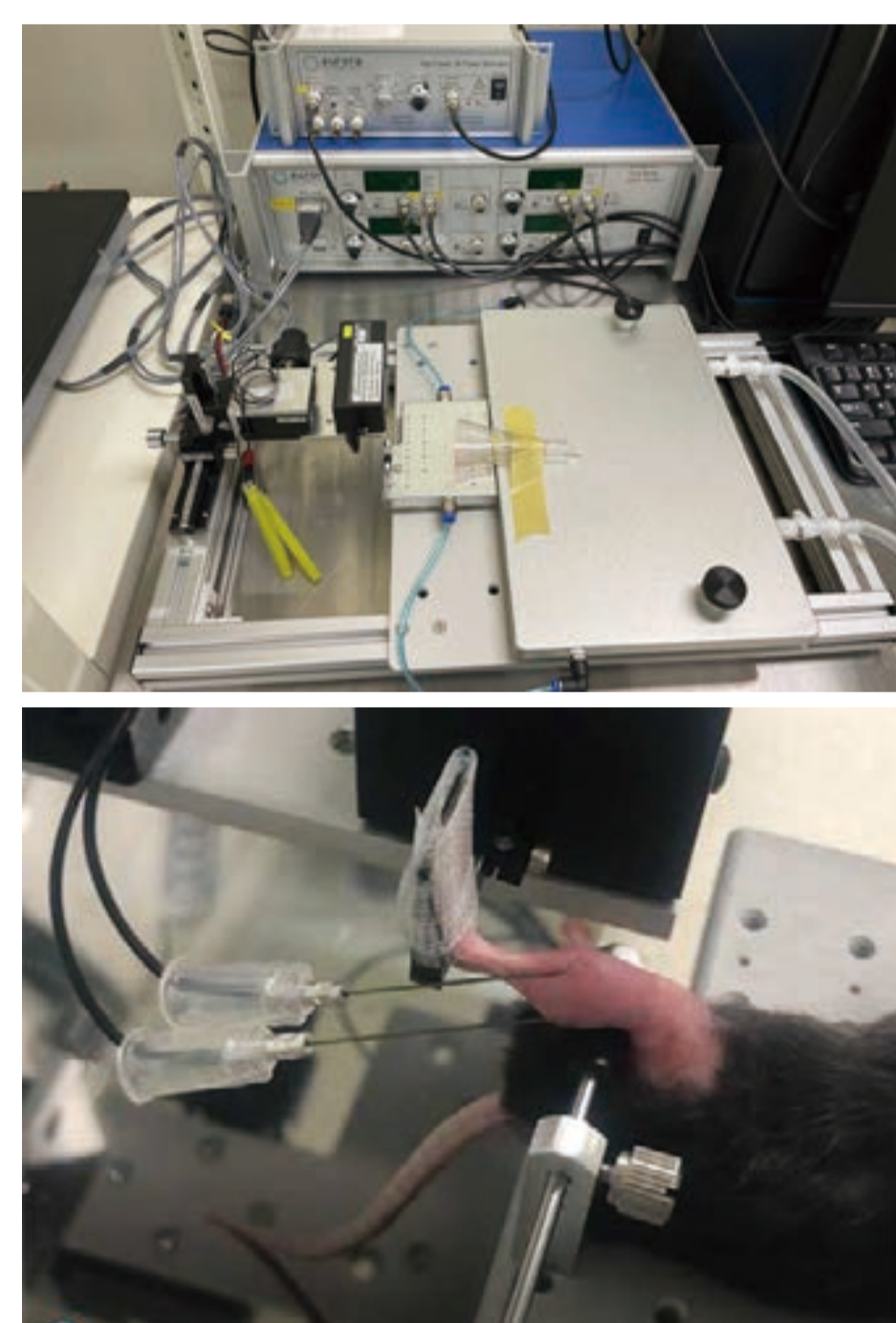
Male mice, C57BL/6J, 13-week-old
Male mice, C57BL/6J-Aged, 41, 54, 80, 93, and 106-week-old
(The Jackson Laboratory Japan, Inc.)

Measurement of muscle strength

In this study, the whole animal muscle test system (1300A, Aurora Scientific) was used for measuring muscle strength. The mice were anesthetized by an isoflurane aspiration and placed in a thermostatically controlled table. The knee is held in place by a bar and the foot firmly fixed to a footplate on the motor shaft. The absolute muscle force were measured by given electrical stimulations at frequency of stimulation (0.35 ms pulse, 100Hz).

Measurement of NAD concentration in skeletal muscle

After euthanasia under isoflurane anesthesia, the gastrocnemius muscle was collected. NAD was extracted from gastrocnemius muscle and quantified by liquid chromatography/mass spectrometry (Triple Q, 6470, Agilent, CA, US.).



The whole animal muscle test system

Experiment 2: Effects of NMN in aged mice

Animals

Male mice, C57BL/6J-aged, 76-week-old (The Jackson Laboratory Japan, Inc.)

Breeding cages

To encourage spontaneous exercise, the animals were kept alone in cages with running wheels. The number of running wheel revolution was measured during the dark period from 7:00 p.m. to 7:00 a.m.

Measurement of muscle strength

The same as Experiment 1.
Measurements were taken before administration (pre), 14 days (2 weeks) and 28 days (4 weeks) after administration.

Administration

Orally administration was started at 76 weeks of age, once a day for 4 weeks.

Group configuration

Group	Age	Dosing solution	Dose level	Dose volume	n
Control	76 weeks	saline	0 mg/kg	5 mL/kg	5
NMN	76 weeks	NMN	300 mg/kg	5 mL/kg	5



The cage with running wheel

Results

Experiment 1

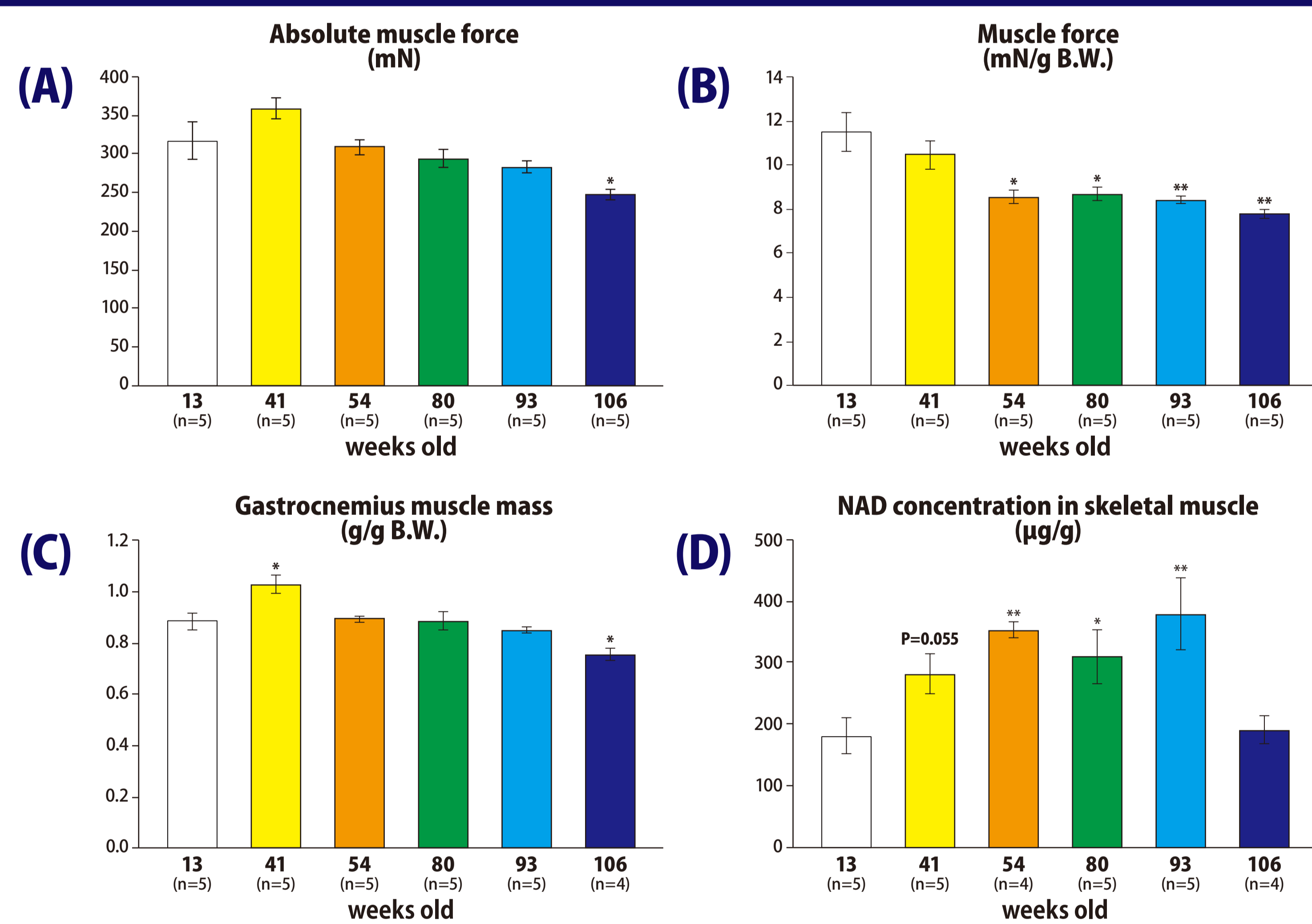


Fig 1 Mean ± S.E. *, p<0.05, **, p<0.01, Significant difference from 13 weeks old group (Student's t-test)

Experiment 2

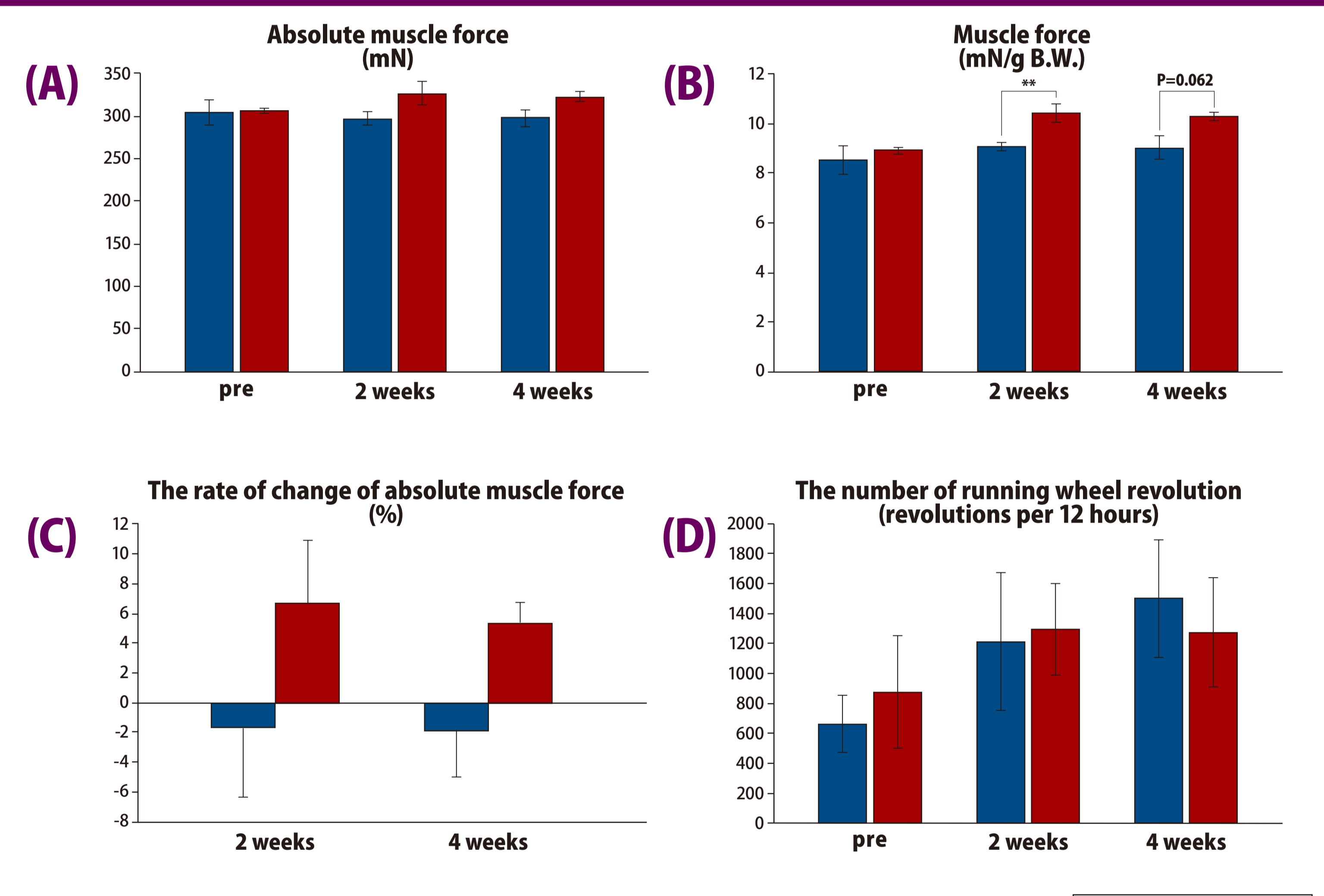


Fig 2 Mean ± S.E. **, p<0.01, Significant difference from control group (Student's t-test)

Conclusion

Experiment 1

- Compared to 13-week-old mice, muscle strength decreased with age, with significant muscle weakness from 54 to 106 weeks of age [Fig 1 (B)].
- Gastrocnemius weight per body weight peaked at 41-week-old mice and decreased with aging. It was significantly lower in 106-week-old mice than 13-week-old [Fig 1 (C)].
- Similar to human sarcopenia, decrease in muscle strength and muscle weight were observed with aging in mice.
- The NAD levels in skeletal muscle were maintained up to 93-week-old mice, but were markedly reduced in 106-week-old mice. Considering the critical role of NAD in maintaining the health of our cells, tissues, and bodies, the NAD levels decrease in skeletal muscle may be one of the causes of age-related muscle weakness [Fig 1 (D)].
- The low NAD levels in 13-week-old mice may be due to the circadian fluctuation of the NAD level.

Experiment 2

- Oral administration of NMN under spontaneous exercise with a running wheel for 4 weeks resulted in an increasing trend in muscle strength [Fig 2 (A), (B), (C)].
- Since there was no significant difference in the number of running wheel revolution in the NMN-treated group compared to the control group, it was suggested that the increase in muscle strength was not due to an increase in spontaneous behavior [Fig 2 (D)].
- It is suggested that the combination of NMN administration and exercise with a running wheel suppresses muscle weakness in aging mice.

Conclusion

- This measurement system was able to detect the decline in muscle strength of aging mice with high sensitivity, and was useful for evaluating changes in mouse muscle strength over time.
- The results of this study was useful to test whether NMN, a type of supplement, reduce muscle loss due to aging.