## 【審查論文】

# The impact of building up the limb muscle mass by the resistance exercise with the intake of milk in middle-aged and elderly women

Michiyo KANEKO, Takehiko KANEKO, Masaki TAGA, Reiko TAKANASHI, Kaori SATO and Shiori TAKANO

## Abstract

Background: In the rapidly aging Japanese society, the most serious disorder to prevent is sarcopenia for extending healthy life expectancy. Objective: This study was carried out to clarify whether five kinds of specific and simple resistance exercise performing at home for the short term (42 days) with the intake of whole milk could help to increase the limb muscle mass in middle-aged and elderly women. Participants and measurements: Subjects were 39 healthy women aged 50 to 80 years who gave the consent to participate in the present study. Body composition, physical fitness, food and nutrient intake were measured. Oral glucose-tolerance test (OGTT) was also performed. Groups and results: Subjects were categorized in two groups; Group I with the increase of the limb muscle mass after exercise and Group II with no increase. Body weight before exercise (Group I,  $51.6\pm5.5$  kg vs Group II,  $58.5\pm10.3$ kg), the body mass index (BMI)  $(21.7 \pm 2.5 \text{ kg/m}^2 \text{ vs } 24.3 \pm 3.9 \text{ kg/m}^2)$ , the limb muscle mass  $(14.4 \pm$ 1.3 kg vs  $15.6\pm2.0$  kg), the skeletal muscle mass index (SMI)  $(6.0\pm0.5$  kg/m<sup>2</sup> vs  $6.5\pm0.7$  kg/m<sup>2</sup>), were all significantly high in Group II. The intake of milk before exercise in Group I (162.1 $\pm$ 103.7 g/day) was significantly higher than in Group II (92.5 $\pm$ 63.3 g/day). The blood glucose level at 30 min after glucose loading in Group I before exercise was significantly higher than that in Group II (199.3  $\pm$  31.1 mg/dl vs 177.0±34.1 mg/dl). Conclusion: The subjects in Group I could successfully increase the limb muscle mass, but the subjects in Group II, whose weight, BMI, the limb muscle mass were significantly high before exercise, could not increase the muscle mass by resistance exercise with the intake of milk. It was considered that the resistance exercise carried out in this study was not strong enough for Group II. We assumed that the BMI value could be the indicator of the strength of exercise for building up the muscle mass in middle-aged and elderly individuals.

**Keywords:** limb muscle mass, BMI, resistance exercise, milk, OGTT

## Introduction

According to the statistics published by World Health Organization in 2018, Japanese average life span, which is 84.2 years old in both sexes, has attained the best longevity in the world<sup>1)</sup>. On the other hand, healthy life expectancy is 74.8 years old<sup>1)</sup>. It means that elderly people live for about ten years with physical and/or mental disorders. In the rapidly aging Japanese society in which the old

people population has been increasing, quantitative and qualitative research for extending healthy life expectancy is critical challenge to improve quality of society.

The most serious disorder to prevent is sarcopenia for extending healthy life expectancy. Sarcopenia, which is defined by the age-dependent loss of skeletal muscle mass and declining muscle function, causes clinical complications including obesity, osteoporosis, type 2 diabetes which could lead to disabilities, the loss of independence, possible mortality in middle-aged as well as elderly individuals<sup>2-6)</sup>. Sarcopenia is also major cause of frailty<sup>6-8)</sup>. Numerous findings have been reported on the assessment and the treatment of sarcopenia<sup>9,10)</sup>, and it is considered that sarcopenia could be prevented or treated by physical activity, nutritional supplementation and drug treatment<sup>11-13)</sup>. Among these treatments, physical activity including resistance exercise was obviously most effective for building up the skeletal muscle mass<sup>14,15)</sup>, but none of these studies showed the impact of specific resistance exercise for the purpose of increasing of muscle mass and function. People did not know what kind of exercise they should carry out to prevent sarcopenia when they intended to start the exercise at home.

Since branched chain amino acids (BCAA) including leucine, valine and isoleucine are main amino acids of skeletal muscle, myofibrillar protein synthesis is regulated by BCAA, especially leucine<sup>16)</sup>. Moreover, the supplementation of BCAA reduced muscle damage induced by exercise and accelerated recovery<sup>17)</sup>. Therefore, it could be considered that the intake of BCAA combined with resistance exercise is effective treatment for muscle synthesis.

In this study, we attempted to show for the first time the impact of five kinds of specific and simple resistance exercise, which people could perform at home and could build up the muscle mass in middle-aged and elderly women. In order to enhance the effect of resistance exercise for building up the skeletal muscle, we asked the subjects to take whole milk which was containing a lot of BCAA. We hope our basic research findings would be beneficial for the treatment and the prevention of sarcopenia among elderly individuals.

## Methods

This study was approved and carried out according to the guidelines of the ethics committee of human research of Wayo Women's University. The subjects gave the written consent to participate in the study. The study was carried out in 2017 and 2018.

## Subjects and measurements

39 healthy middle-aged and elderly women aged 50 to 80 years ( $64.5\pm8.8$  years) who lived in Chiba prefecture and Tokyo were enrolled in the present study. Since the study by Tanimoto et al. showed that the limb muscle mass in women decreased dramatically from around 50 years, we recruited the subjects ranged from 50 to  $80^{18}$ ). Individuals who were getting the treatment of diabetes were excluded. Body composition including muscle mass, estimated bone mass, body fat mass was measured by impedance method (MC-190, TANITA Corp., Tokyo, Japan). The value of BMI and SMI were calculated. Grip strength was measured by the squeeze dynamometer. Walking speed was measured when subjects walked 10m at the usual walking speed. We counted how many times subjects could stand up from the 40 cm chair for 30 second, this measurement is generally called "stand up test".

## Exercise and the intake of milk

Participants carried out the following resistance exercises for 42 days every day, 1) slow squat using a chair, 2) lifting each knee  $20 \sim 30$  cm slowly at the position of sitting on the chair, 3) stretching each knee to the level of horizontal position slowly and then bending it at the position of sitting on the chair, 4) inner thigh exercise; putting a 20 cm exercise ball between knees and squeezing it slowly and then relaxing at the position of sitting on the chair, 5) exercise for the back side of thigh; putting the exercise ball under the heel and pushing it down slowly and then relaxing at the position of extending knees on the mat. Each exercise was carried out 10 × 2 times a day. Subjects took 200ml of whole fat cow milk after exercise. The period of exercise was decided following the preceding study<sup>19)</sup>.

## Glucose-tolerance test

Standard 75-g oral glucose-tolerance test (OGTT) was performed at the fingertip, and capillary glucose values were measured at fasting without any food for 10 hours before measurement, and 30, 60, and 120 min after taking 75g glucose in 225 ml water.

#### Food and nutrient intake

Brief-type self-administered diet history questionnaire (BDHQ) was conducted for analyzing food and energy intakes<sup>20-22)</sup>. The subjects were requested to answer the questionnaires which contained the kinds of food, amount and the frequency the subjects had for the previous one month of the investigation. 4 page sheets filled by each subject were sent to the EBN JAPAN (DHQ Support Center, Tokyo, Japan) which analyzed energy intake, nutrient intake, kind of food and amount of food for one month.

## Statistical analysis

Body composition, food and nutritional values and physical fitness values were compared between the two groups. Two-tailed student's t-test, followed by the F-test checking variance, was performed. Two-tailed paired student's t-test was performed when the values were compared between before and after exercise in a group. Statistical analysis in OGTT was carried out also by two-tailed student's t-test. p<0.05 was considered significant difference.

## Results

## Body composition and physical activities

The values of body composition of all the subjects were compared between before and after exercise, but there was no significant difference. So the subjects were categorized in two groups; Group I  $(65.6\pm9.3$ years old, n=16) with increase of the limb muscle mass, and Group II (63.9 $\pm$ 8.5 years old, n=23) with no increase of the limb muscle mass. The values of body composition and physical fitness in Group I were compared between before and after exercise shown in Table 1. The values of weight after exercise, right arm muscle, left arm muscle, right leg muscle, left leg muscle, limb muscle mass, SMI were significantly higher than those before exercise. The values of walking speed and stand up test were also significantly higher after exercise than before exercise.

Table 1 Body composition and physical fitness compared between before and after exercise in Group 1

	Before exercise	After exercise
Weight (kg)	51.6 ± 5.5	52.2 ± 5.3*
Body fat (kg)	$15.2 \pm 4.1$	$15.2 \pm 4.0$
BMI (kg/m <sup>2</sup> )	$21.7 \pm 2.5$	$21.8 \pm 2.5$
Trunk muscle (kg)	$19.93 \pm 1.44$	$20.13 \pm 1.38$
Right arm muscle (kg)	$1.58 \pm 0.15$	$1.63 \pm 0.16^*$
Left arm muscle (kg)	$1.51 \pm 0.15$	$1.55 \pm 0.16^*$
Right leg muscle (kg)	$5.69 \pm 0.54$	$5.83 \pm 0.45^*$
Left leg muscle (kg)	$5.59 \pm 0.55$	$5.76 \pm 0.46^*$
Limb muscle mass (kg)	$14.37 \pm 1.30$	$14.77 \pm 1.14^*$
SMI (kg/m²)	$6.0 \pm 0.5$	$6.2 \pm 0.5^*$
Grip strength (kg)	$24.6 \pm 3.9$	$24.5 \pm 4.3$
Walking speed (m/sec)	$1.3 \pm 0.2$	$1.4 \pm 0.2^*$
Stand up test (times/30sec)	$18.4 \pm 4.2$	$25.9 \pm 6.0^*$

Values are means ±SD

BMI; body mass index

SMI; skeletal muscle mass index

As shown in Table 2, the values of body compositions before exercise were compared between the two groups in order to clarify what was the difference in body composition for increasing the muscle mass. Weight (Group I,  $51.6 \pm 5.5$  kg vs Group II,  $58.5 \pm 10.3$  kg), body fat  $(15.2 \pm 4.1$  kg vs  $20.1 \pm 7.9$  kg), BMI  $(21.7 \pm 2.5$  kg/m² vs  $24.3 \pm 3.9$  kg/m²), the limb muscle mass  $(14.4 \pm 1.3$  kg vs  $15.6 \pm 2.0$  kg), SMI  $(6.0 \pm 0.5$  kg/m² vs  $6.5 \pm 0.7$  kg/m²), were significantly greater in Group II than in Group I. The value of estimated bone mass before exercise  $(2.0 \pm 0.2$  kg vs  $2.2 \pm 0.3$  kg), grip strength  $(24.6 \pm 3.9$  kg vs  $24.2 \pm 4.0$  kg), walking speed  $(1.3 \pm 0.2$  m/sec vs  $1.3 \pm 0.2$  m/sec), stand up test  $(18.4 \pm 4.2$  times/30sec vs  $17.7 \pm 4.2$  times/30sec), showed no significant difference between the two groups.

Table 2 Body composition and physical fitness in the two groups before exercise

	Group I (n=16)	Group II (n=23)
Age	65.6 ± 9.3	63.9 ± 8.5
Height (cm)	$154.4 \pm 6.3$	$155.1 \pm 5.0$
Weight (kg)	$51.6 \pm 5.5$	$58.5 \pm 10.3^*$
Body fat (kg)	$15.2 \pm 4.1$	$20.1 \pm 7.9^*$
BMI (kg/m²)	$21.7 \pm 2.5$	$24.3 \pm 3.9^*$
Limb muscle mass (kg)	$14.4 \pm 1.3$	$15.6 \pm 2.0^*$
SMI (kg/m <sup>2</sup> )	$6.0 \pm 0.5$	$6.5 \pm 0.7^*$
Estimated bone mass (kg)	$2.0 \pm 0.2$	$2.2 \pm 0.3$
Grip strength (kg)	$24.6 \pm 3.9$	$24.2 \pm 4.0$
Walking speed (m/sec)	$1.3 \pm 0.2$	$1.3 \pm 0.2$
Stand up test (times/30sec)	$18.4 \pm 4.2$	$17.7 \pm 4.2$

Values are means±SD

\*; significant difference (p<0.05)

BMI; body mass index SMI; skeletal muscle mass index

# Analysis of food and nutrient intake

Nutrient intakes before exercise were shown in Table 3. The intake of carbohydrate (Group I, 251.9  $\pm\,77.8$  g/day vs Group II, 232.5  $\pm\,71.9$  g/day), calcium (687.2  $\pm\,257.6$  mg/day vs 637.6  $\pm\,312.8$  mg/day), cholesterol (532.0  $\pm\,245.4$  mg/day vs 489.5  $\pm\,185.1$  mg/day), retinol (541.0  $\pm\,300.0$  µg/day vs 519.3  $\pm\,264.3$  µg/day), and vitamin C (170.2  $\pm\,50.9$  mg/day vs 144.8  $\pm\,71.8$  mg/day) were slightly

<sup>\*;</sup> significant difference (p<0.05)

higher in Group I than in Group II, though there was no significant difference. Food intakes before exercise were compared between the two groups (Table 4). The intake of tofu • fried tofu  $(59.5\pm50.3)$  g/day vs  $67.0\pm52.8$  g/day), green vegetable  $(41.5\pm24.3)$  g/day vs  $52.3\pm48.1$  g/day) were higher in Group II than in Group I, but there was no significant difference. The intake of whole fat milk in Group I  $(162.1\pm103.7)$  g/day) was significantly higher than that in Group II  $(92.5\pm63.3)$  g/day).

## Glucose metabolism

OGTT was performed before exercise, and the glucose values at the fingertip were measured at fasting, 30, 60, and 120 min after glucose loading (Figure 1). The blood glucose level at 30 min in Group I (199.3  $\pm$  31.1 mg/dl), showing the steep increase of blood glucose level from the fasting level, was significantly higher than in Group II (177.0 $\pm$ 34.1 mg/dl). At 60 min, it was higher in Group I than in Group II (198.8 $\pm$ 56.8 mg/dl vs 180.0 $\pm$ 42.6 mg/dl), though there was not significant difference. Blood glucose values at fasting in both groups (106.7 $\pm$ 11.7 mg/dl vs 105.6 $\pm$ 11.5 mg/dl) were similar. The value at 120 min post-loading in Group I was 171.4 $\pm$ 52.0 mg/dl and 153.2 $\pm$ 32.1 mg/dl in Group II, there showed no significant difference.

Table 3 Nutrient intake compared between the two groups before exercise

	Group I	Group II
Energy intake (kcal/day)	$2010.5 \pm 610.2$	$1919.1 \pm 515.2$
Protein (g/day)	$83.9 \pm 31.5$	$83.2 \pm 28.9$
Fat (g/day)	$68.5 \pm 24.1$	$65.2 \pm 18.8$
Carbohydrate (g/day)	$251.9 \pm 77.8$	$232.5 \pm 71.9$
Mineral (g/day)	$21.9 \pm 6.3$	$21.4 \pm 6.8$
Calcium (mg/day)	$687.2 \pm 257.6$	$637.6 \pm 312.8$
Dietary fiber (g/day)	$15.0 \pm 4.9$	$14.4 \pm 5.6$
Cholesterol (mg/day)	$532.0 \pm 245.4$	$489.5 \pm 185.1$
Retinol (μg/day)	$541.0 \pm 300.0$	$519.3 \pm 264.3$
VitaminB <sub>1</sub> (mg/day)	$1.0 \pm 0.3$	$0.9 \pm 0.3$
VitaminB <sub>2</sub> (mg/day)	$1.7 \pm 0.5$	$1.5 \pm 0.5$
Vitamin C (mg/day)	$170.2 \pm 50.9$	$144.8 \pm 71.8$
Vitamin D (ug/day)	$18.6 \pm 12.5$	$19.2 \pm 14.8$

Values are means  $\pm$  SD

Table 4 Food intake compared between the two groups before exercise

	Group I	Group II
Rice (g/day)	$195.8 \pm 109.6$	$196.4 \pm 101.7$
Beef • pork (g/day)	$45.4 \pm 25.5$	$44.8 \pm 22.1$
Egg (g/day)	$57.8 \pm 35.6$	$47.7 \pm 20.1$
Tofu · fried tofu (g/day)	$59.5 \pm 50.3$	$67.0 \pm 52.8$
Green vegetable (g/day)	$41.5 \pm 24.3$	$52.3 \pm 48.1$
Mushroom (g/day)	$15.3 \pm 7.4$	$16.0 \pm 9.1$
Seaweed (g/day)	$16.6 \pm 12.4$	$15.6 \pm 14.6$
Regular milk (g/day)	$162.1 \pm 103.7$	$92.5 \pm 63.3^*$

Values are means±SD

<sup>\*;</sup> significant difference (p<0.05)

 $<sup>^*</sup>$ ; significant difference (p<0.05)

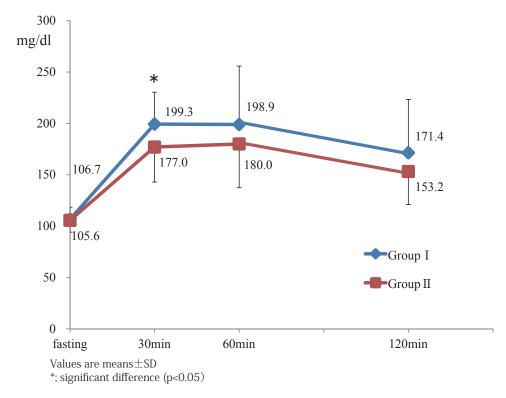


Figure 1 OGTT compared between the two groups before exercise

## Discussion

We attempted to examine whether five kinds of specific resistance exercise which everybody could carry out at home and taking whole milk for 42 days could increase the skeletal muscle mass for the prevention of sarcopenia in middle-aged and elderly women. The subjects in Group I could successfully build up the limb muscle mass by the exercises with milk. The values of weight before exercise, body fat, BMI, the limb muscle mass, and SMI in Group I were significantly lower than those in Group II. In Group I, the amount of the limb muscle mass was considered to contribute the increase of weight because body fat did not change after exercise. The average value of BMI in Group I was 21.7 kg/m² which represented normal body shape. These results suggested that the exercise carried out in our study was appropriate strength to increase the limb muscle for the subjects with the normal range of BMI as in Group I. Then, why didn't the limb muscle mass increase in Group II? The muscle mass and the BMI value before exercise in Group II were higher than those in Group I. The exercise might have been not strong enough for the subjects in Group II whose average BMI was 24.3 kg/m² and muscle mass was relatively high. We would say that the stronger exercise had to be designed for the subjects with higher BMI, and the strength of exercise should be decided depending on the BMI value to enhance the efficacy for building up the muscle mass.

Since the low skeletal muscle mass is strongly associated with an increase in type 2 diabetes  $risk^{23}$ , we carried out OGTT to examine the relationship between the muscle mass and the function of glucose regulation. In general, the definition of diabetes is that glucose level at 120 min after glucose loading is over 200 mg/dl. In our results, glucose level measured at 120 min in Group I was 171.4 mg/dl, and they would not be diagnosed diabetes. However, the blood glucose level of Group I at 30 min was significantly

high showing steep increase from 106.7 mg/dl at fasting to 199.3 mg/dl. Some reports have shown that the large fluctuation of blood glucose level impaired endothelial function and the acute glucose fluctuations triggered oxidative stress leading to atherosclerosis<sup>24-26)</sup>. It was also reported that the higher skeletal muscle mass enhanced the peripheral glucose utilization because of the greater expression of GLUT4 which was the glucose transporter regulated by insulin<sup>27)</sup>. In our study, the glucose regulation in Group I was considered to be exacerbated by the less muscle mass. These findings suggested that the less muscle mass in Group I would restrict the glucose utilization resulting in the steep rise of blood glucose level at 30 min. They also suggested that the big swing from the level of fasting to 30 min would accelerate atherosclerosis leading to potential mortal cardiovascular diseases. Our results indicated that the subject in Group I should build up the muscle mass to reduce the risk of not only sarcopenia, but also to reduce the risks of serious clinical disorders including type 2 diabetes and atherosclerosis leading to mortality in their later lives.

Some studies reported that the intake of milk after resistance exercise stimulated myofibrillar protein synthesis in the skeletal muscle<sup>28,29)</sup>. As Granic A. et al. showed the feasibility and acceptability of whole or skimmed milk with resistance exercise for preventive measure to preserve muscle health and function in elderly population, it was considered that the intake of whole milk after resistance exercise contributed to build up the skeletal muscle<sup>30)</sup>. On the basis of these findings, we asked the subjects to take 200 ml milk following the resistance exercise in order to stimulate the increase of the skeletal muscle mass. As we showed in the results of food intake, the intake of whole fat milk in Group I before exercise was significantly higher than that in Group II. The limb muscle mass in Group I might have increased due to the habit of drinking milk.

We showed in this study that the specific resistance exercise performing at home with the intake of milk for 42 days increased the limb muscle mass in some subjects (Group I), but did not increase in other subjects (Group II). Weight, body fat, BMI, the limb muscle mass, SMI before exercise were significantly high in Group II. It was considered that the exercise carried out in this study was not strong enough for the subjects in Group II. Since the BMI value before the beginning of exercise was significantly high in Group II, it could be the indicator for the decision of the strength of exercise to enhance the efficacy of building up the limb muscle mass. The blood glucose level at 30 min after glucose loading in Group I was significantly higher than that in Group II, which showed the reduced regulation of glucose level due to the less muscle mass in Group I.

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Michiyo Kaneko (Wayo Women's University, Department of Nursing)

Takehiko Kaneko (Wayo Women's University, Department of Health and Nutrition)

Masaki Taga (Wayo Women's University, Department of Health and Nutrition)

Reiko Takanashi (Wayo Women's University, Graduate School of Life Sciences)

Kaori Sato (Wayo Women's University, Department of Health and Nutrition)

Shiori Takano (Wayo Women's University, Graduate School of Human Ecology)

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