Obesity is associated with lifestyle habits such as inactivity, bad eating practices, stress and excessive net intake of calories (1). Among these, energy imbalance and inactivity are becoming serious problems. Total energy cost can be divided into three parts, including resting metabolic rate (RMR), diet-induced thermogenesis (DIT), and the amount of energy used for physical activity energy expenditure (AEE) (2). The greatest component of daily energy expenditure is RMR, constituting about 70% of the total energy expenditure (3, 4).

Silk peptides (SP) have been ingested for a long time in Asian countries (5). In recent years, additional applications have been developed for silk, mainly in the field of biotechnology and biomedicine. The versatility of these new implementations arises from the singularity of the molecular structure of silk (6). Proteins such as fibroin and sericin are the main constituents of silk, with fibroin constituting 70 to 80% and sericin 20 to 30% of the total cocoon weight (6, 7).

Many studies regarding the health benefits of SP intake have been reported (8–11). SP not only regulate blood glucose level and hyperlipidemia but also increase tissue injuries and increasing anti-fatigue (14). Moreover, it is well known that increasing the concentration of leptin leads to an exothermic reaction and an increase in the amount of activity. Therefore, SP promotes fat oxidation and increases energy expenditure (15, 16). Additionally, in several rat and mouse model studies, food intake and body weight were decreased in rodents fed ad libitum (5, 14, 16).

However, measurements in those studies have been conducted for analysis of blood profiles and hormones in mice. Accordingly, we investigated the effects of SP administration using an open circuit calorimetry system on resting energy expenditure and substrate utilization in resting mice for the duration of 24 h. Seven-week-old male ICR-mice were orally administered SP (800 mg/kg) for 2 wk and were subjected to endurance training. The results indicated that not only was oxygen uptake higher in the SP group than in the CON group (*p<0.05), but also the respiratory exchange rate was lower than that in the CON group for the duration of 24 h (**p<0.01). Moreover, fat oxidation was increased in the SP group. Body weight of the SP group was significantly decreased compared to that of the CON group (*p<0.05). These results suggest that intake of silk peptides increases fat oxidation during rest in exercised mice. Intake of silk peptides is considered to be a favorable supplement for athletes in training. In particular, it would be an effective supplement for athletes who require weight loss along with an increase in muscle mass.

Key Words silk peptides, FER, RMR, fat oxidation

Summary Silk peptides (SP) have been reported to decrease body weight and accumulate fat. We investigated the effects of SP administration by using an open circuit calorimetry system on resting energy expenditure and substrate utilization in resting mice for the duration of 24 h. Seven-week-old male ICR-mice were orally administered SP (800 mg/kg) for 2 wk and were subjected to endurance training. The results indicated that not only was oxygen uptake higher in the SP group than in the CON group (*p<0.05), but also the respiratory exchange rate was lower than that in the CON group for the duration of 24 h (**p<0.01). Moreover, fat oxidation was increased in the SP group. Body weight of the SP group was significantly decreased compared to that of the CON group (*p<0.05). These results suggest that intake of silk peptides increases fat oxidation during rest in exercised mice. Intake of silk peptides is considered to be a favorable supplement for athletes in training. In particular, it would be an effective supplement for athletes who require weight loss along with an increase in muscle mass.

Key Words silk peptides, FER, RMR, fat oxidation

Materials and Methods

Animals. Seven-week-old male ICR-mice (n=18) were used. Mice were purchased from Orient Bio Inc. (Seongnam, Korea). All mice were housed in standard plastic cages under controlled conditions of humidity (50%) and temperature (23±1°C) with alternating 12 h cycles of light and dark. Mice were adapted to the laboratory housing conditions for 3 d. They were given free access to water and a non-purified commercial diet (5L79, Orient Bio Inc.) containing (g/kg diet): crude protein, 180; crude fat, 52; crude fiber, 52; minerals, 57; and carbohydrate, 368. The protein, fat, and carbohydrate ratio (%) based on calories was 21 : 14 : 65, and

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gross and metabolizable caloric contents of the diet were 4.04 and 3.21 kcal/g, respectively. All experimental procedures were carried out at the Animal Experiment Research Center of Konkuk University. This study was conducted in accordance with the ethical guidelines of the Konkuk University Institutional Animal Care and Use Committee.

Silk peptides. Silk peptides (SP) were obtained from Worldway Co., Ltd (Jeoneui, Korea). SP are mainly composed of Ala (34.36%) > Gly (27.23%) > Iso (15.51%) > Ser (9.58%) and minor amino acids. Details on the composition are shown in Table 1. The composition of silk peptides by molecular weight was as follows: approximately 150–350 D and an average molecular weight of about 250 D. SP were dissolved in distilled water and administered to the SP group at 800 mg/kg; the CON group was administered distilled water orally every day and then received training for 2 wk (14).

Training methods. Exercise was carried out at a certain period at a frequency of five times a week for a total of 2 wk. After 3 d of adaptation to the treadmill, the intensity of the exercise was held steady by maintaining the slope at an 8˚ incline (17). Details on the exercise program are shown in Table 2.

Measurement of resting metabolic rate. RMR was measured before and after SP ingestion for 2 wk. Two hours before the measurement, mice were put in a metabolic chamber to reduce stress. The flow rate was kept constant at 1.2 L/min and measured for 24 h (17). A non-purified commercial diet and water were given freely to the mice.

Respiratory gas analysis. Respiratory gas was measured using an open-circuit device based on previous studies (4, 17). Oxygen uptake and CO_2 production were measured using a mass analyzer (model RL-600, Alco System, Chiba, Japan; used as a gas analyzers), a switching system, and a gas analyzer. Following this, the respiratory exchange ratio, amount of carbohydrate oxidation, and amount of fat oxidation were calculated. Air from each chamber was passed through an acrylic tube of about 6 mm in diameter and 3 m in length and sampled for 60 s.

Statistical analyses. Data are given as mean ± SD. All statistical analyses were performed with SPSS version 19.0 software (SPSS, Inc., Chicago, IL, USA). Respiratory gas analysis provided the necessary data for the statistical analysis. The significance of differences between groups was determined using an ANOVA test with a post hoc Tukey test. The significance level was set at p < 0.05.

Table 1. Amino acid compositions (%) of SP.

| Amino acid | SAA-1 |
|------------|-------|
| Asp        | 1.68  |
| Ser        | 9.58  |
| Glu        | 1.28  |
| Gly        | 27.23 |
| His        | 0.21  |
| Arg        | 0.17  |
| Thr        | 2.00  |
| Ala        | 34.36 |
| Pro        | 0.44  |
| Iso        | 15.51 |
| Cys        | 0.05  |
| Tyr        | 0.41  |
| Val        | 3.49  |
| Met        | 0.10  |
| Lys        | 0.10  |
| Ile        | 1.25  |
| Leu        | 1.24  |
| Phe        | 0.87  |
| Trp        | 0.05  |

Table 2. Training method.

| Training | Constant angle | Speed/running time |
|----------|----------------|--------------------|
| 1 wk     | 8%             | 20 m/min, 50 min   |
| 2 wk     | 8%             | 25 m/min, 50 min   |

Fig. 1. Changes of body weight in the CON and SP groups for 2 wk. *p<0.05, ***p<0.001.
tory gas, oxygen uptake, RER, fat oxidation, food intake and body weight were analyzed by two-way repeated measures analysis of variance. Test periods were compared by the t-test. Differences were considered significant at $p<0.05$.

**Results and Discussion**

**Weight change and food intake**

Changes in weight during the 2 wk of the observational period are shown in Fig. 1. Weights of mice increased significantly throughout the experiment period (**$p<0.001$), reaching 36.7±2.2 g in the CON group and 35.4±1.5 g in the SP group. In addition, that of the SP group significantly decreased compared to that of CON group (*$p<0.05$). Recent studies have reported that silk peptide ingestion is effective in weight loss for white mice (5, 16, 18). In the present study, a significant weight loss was also exhibited in the group with 2 wk of exercise and silk peptide intake (800 mg/kg) compared to that of the control group. According to previous studies, weight loss occurs because aerobic exercise increases energy expenditure along with synergistic effects of the silk peptides (19). In the present study, a significant weight loss was also considered to be due to the increase of energy expenditure caused by 2 wk of silk peptide intake and synergistic effects of the exercise.

Nonetheless, 2 wk of dietary intake showed a significant increase compared to that of the control group. Food intakes in the CON and SP groups were 4.38±0.58 g/d and 4.80±0.50 g/d during the 2 wk of the experiment, respectively. The total amount of food intake during the 2 wk was significantly higher in the SP group than that in the CON group (**$p<0.001$, Table 3). On the other hand, the food efficiency ratio was higher in the CON group than that in the SP group (**$p<0.001$, Table 3). It is known that silk peptides elevate exothermic reactions and physical activities while decreasing dietary intake, thus eventually increasing the concentration of leptin to result in reduction of body weight and body fat which are well associated with obesity (16, 18). The main signals of leptin maintain nutritional status, dietary intake regulation, and energy balance via the hypothalamus (20, 21). Lee et al. (15) have reported that silk peptides reduce the increased rate of blood glucose level by stimulating insulin secretion and increasing the serum leptin level in OLETF rats. Additionally, when blood leptin concentration was measured in an insulin-dependent diabetes rat model (STZ-induced diabetes), no statistical difference was observed in the normal control group or in the diabetes control group whereas a significant increase was detected in the concentration of the silk peptide treatment group (18). It is reported that the intake of silk peptides reduces the dietary intake of high fat diet induced-rats by 20% compared to that of a control group (5). In contrast, Hwang et al. (12) found no changes in dietary intake. Meanwhile, leptin is delivered to the hypothalamus via binding with blood receptors and decreases as the ratio of body fat increases. It has also been observed that the elevation of energy expenditure by exercise directly reduces the synthesis and secre-

| Table 3. Food intake (FI) and food efficiency ratio (FER) in CON and SP groups. |
|---------------------------------|------------------|-------------------|
| FI (g/d) | FER |
| CON | 4.38±0.58 | 0.6±0.1 |
| SP | 4.83±0.55*** | 0.2±0.03*** |

***$p<0.001$. 

Fig. 2. Changes of oxygen uptake of Pre (A), Post (B) and Sum (C) in the CON and SP groups for 24 h according to RMR measurements. ***$p<0.001$, N.S., no significance.
tion of leptin (22, 23). In this study, the SP group showed a significant increase in dietary intake compared to that of the control group. This result may be caused by the increased action of leptin by silk peptides as well as metabolic homeostasis as VO2max 75% of aerobic exercise increases. Moreover, the result implies that application of various methods is warranted in future studies.

Resting metabolic rate (RMR)

Oxygen uptake of Pre (non-intake) and Post (intake) showed changes over time (***p<0.001; Fig. 2A and B). However, it was exhibited that 24 h of oxygen uptake (%) significantly increased in the SP group compared to the CON group (Fig. 2C). Meanwhile, no significant difference was observed in the CON group with 2 wk of endurance exercise and intake of distilled water regarding the resting energy metabolism. This finding is in agreement with a study performed by Jeon et al. (17); they concluded that VO2max 75% of 4-wk treadmill exercise was not sufficient to elevate the resting energy metabolism. Moreover, the result of this study is also in agreement with other studies that have addressed the fact that endurance exercise increases the energy expen-

Fig. 3. Changes of respiratory exchange ratio of Pre (A), Post (B) and Mean (C) in the CON and SP groups for 24 h according to RMR measurements. ***p<0.001, **p<0.01, N.S., no significance.

Fig. 4. Changes of fat oxidation of Pre (A), Post (B) and Sum (C) in the CON and SP groups for 24 h according to RMR measurements. ***p<0.001, **p<0.01, N.S., no significance.
titure of skeletal muscles while it does not have any significant direct effect on 24-h total energy metabolism (24, 25).

Respiratory exchange rate, calculated by the ratio of oxygen uptake and carbon dioxide production, was shown to change over time between Pre (non-intake) and Post (intake) (**p<0.001; Fig. 3A and B). However, it was observed that 24 h of mean RER was significantly decreased in the SP group (**p<0.01; Fig. 3C).

In addition, fat oxidation was also calculated from VCO₂ and VO₂ values, and it followed a circadian rhythm for over 24 h in both groups (p<0.001; Fig. 4A and B). However, the sum of fat oxidation over 24 h was higher in the SP group than that in the CON group (**p<0.01; Fig. 4C).

Unfortunately, we could not find a previous report on RMR in mice for the purpose of comparison. However, energy expenditure seems to increase considering the reduction of accumulated body fat (5, 16). Lee et al. (5, 26) have reported that SP intake inhibited adipogenesis of adipocytes by blocking adipogenic gene expression and protein synthesis. Moreover, reduction of fat weight was due to the lowered amount of fat tissue as well as decreased size of adipocytes, which was confirmed from MRI analysis on abdominal fat distribution.

A previous study reported that SP intake for 30 d showed enhanced swimming ability through recovery of exercise-induced tissue injury and energy depletion (14). Furthermore, Shin et al. (19) have reported that SP not only enhance physical stamina by minimizing damage to tissues, including muscles, but also prevent energy (glycogen) depletion caused by swimming stress. Moreover, tissue glycogen was well preserved by the branched-chain amino acids, although there were controversial results on the role of glycogen. Given the above results, it was suggested that silk peptide intake increases resting energy metabolism in white mice, with particular increase in fat oxidation.

Nevertheless, in our study, we did not use untrained mice as the control group. This is because we 1) intended to differentiate our study from previous studies, and 2) wanted to prove the effects of supplementation of silk peptides in exercise training. Taken together, it seems that 2 wk of exercise and silk peptide intake increase resting energy expenditure and induce fat oxidation. It appears that such silk peptides are responsible for induction of fat oxidation during training; in particular, it is considered to be an effective supplementation for athletes who need to achieve body weight loss and muscle mass increment simultaneously as well as for normal subjects who need to reduce body weight. Thus, in future investigations, it is necessary to understand the effects of long-term intake of silk peptides and training on either resting or exercise energy metabolism in humans.

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