Provision of a voluntary exercise environment enhances running activity and prevents obesity in Snark-deficient mice

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Snark to an environment that allows voluntary exercise promotes increased voluntary running activity; adipose tissue; adipokine/H11032 Snark; adenosine 5

In addition to the regulation of cellular energy balance under various types of stress, such as nutrient deprivation, ischemia, and exercise at the tissue and whole body levels (3, 10, 12). In addition to the regulation of cellular energy balance increases. We expected that Snark-deficient mice would demonstrate less running activity because of their lower oxygen consumption. Surprisingly, however, Snark-deficient mice ran greater distances than their wild-type counterparts and exhibited less fat deposition. Our results indicated that exposure to
an environment that allows voluntary exercise promotes increased running activity and prevents obesity in Snark-deficient mice. To our knowledge, this is the first report of an effect of SNARK on physical activity levels in vivo.

MATERIALS AND METHODS

Animals and treatments. All experimental protocols were approved by the institutional ethics review committee of the Juntendo University and the National Cancer Center Hospital East. The experiments were performed according to the American Physiological Society Guiding Principles in the Care and Use of Animals.

First, to examine the initial endurance capacity of untrained mice, incremental treadmill running (all-out test) was performed. After the 10-min acclimation (1 day), sedentary 2-mo-old male mice (Snark<sup>+/+</sup>; n = 8; Snark<sup>−/−</sup>; n = 8) underwent treadmill running (grade: 10%; initial speed: 10 m/min; increment in speed: 1 m/min) until exhaustion. The time to reach exhaustion was measured.

To examine the acute effects of voluntary running, a short-term experiment was performed. Female mice (n = 32; 3 mo old) were assigned to sedentary or exercise groups. Mice in the exercise group (Snark<sup>−/−</sup>/ExS: n = 8; Snark<sup>−/−</sup>/ExC: n = 8) were housed in individual cages containing an exercise wheel (WW3202; Ohara, Tokyo, Japan), and mice in the sedentary group were housed in individual cages without an exercise wheel (Snark<sup>−/−</sup>/CIS: n = 8; Snark<sup>−/−</sup>/CIL: n = 8). The voluntary wheel-running system consisted of a wheel 14.5 cm in diameter with a running surface 5.0 cm wide, equipped with an optical sensor that was activated by wheel rotation. After a 2-day preexercise period, voluntary running was allowed for 1 wk, and the running distance was recorded every day. As an index of body temperature, colonic temperature was measured using a calibrated thermistor probe 6 h after the beginning of the light period every day until 1 wk after voluntary running as the postexercise period. Mice were maintained under a 12:12-h light-dark cycle with ad libitum access to food and water and standard chow diet (23 ± 1°C room temperature, 55 ± 5% humidity). Food intake was also determined and recorded.

The long-term experiment was performed to clarify the differences in voluntary running activity and its effect between genotypes more. Male mice (n = 32; 2 mo old) were assigned to sedentary or exercise groups. As in the short-term experiment, mice in the exercise group (Snark<sup>−/−</sup>/ExL: n = 8; Snark<sup>−/−</sup>/ExY: n = 8) and the sedentary group (Snark<sup>−/−</sup>/CIL: n = 8; Snark<sup>−/−</sup>/CIL: n = 8) were housed in individual cages with/without an exercise wheel. In addition, 2-mo-old male mice (Snark<sup>−/−</sup>/CILY: n = 5; Snark<sup>−/−</sup>/CILY: n = 5) were assigned to a young sedentary preexercise control group. Mice were maintained under a 12:12-h light-dark cycle with ad libitum access to water and standard chow diet (23 ± 1°C room temperature, 55 ± 5% humidity). After a 1-wk acclimation period, the running distance was recorded every 6 s for each animal throughout the duration of the exercise period. Food intake was also determined and recorded weekly. The end of the exercise period was decided when an apparent difference in running activity was observed between the genotypes (5 mo).

Sample collection. Two weeks after the first exercise period of the short-term experiment, mice were placed in the voluntary running environment for 3 days (3 mo of age), and it was confirmed that running activity and body temperature levels were increased, as in the first exercise period. The mice were then killed, and liver, kidney, white adipose tissue (WAT; gonadal fat), and brown adipose tissue (BAT; collected from between the shoulder blades) were dissected and weighed. Blood samples were also taken, and serum was collected using BD Microtainer serum separators (BD, Franklin Lakes, NJ). Tissue and serum samples were frozen in liquid nitrogen and stored at −85°C until analysis.

For the long-term experiment, animals in the exercised condition were killed after the end of the exercise period (7 mo of age). Sedentary control mice were also killed at 7 mo of age, although young sedentary preexercised control mice were killed at 2 mo of age. Heart, liver, kidney, WAT, BAT, extensor digitorum longus, tibialis anterior, soleus, gastrocnemius, and plantaris muscles on the left side were dissected out and weighed. The triceps surae muscle (gastrocnemius, soleus, and plantaris muscles) on the contralateral side was collected for histological analysis. Tissue samples were frozen in liquid nitrogen and stored at −85°C until analysis.

Histological analysis. Histological samples were embedded in Tissue-Tek OCT compound (Sakura Finetek Japan, Tokyo, Japan), and then transverse sections 10 μm thick were cut using a microtome (CM3050S; Leica, Wetzlar, Germany) at −24°C and mounted on glass slides. ATPase staining (preincubation, pH 4.3) was performed for classification of muscle fiber type. Sections were examined under a light microscope (IM500; Leica), and images were captured with a digital camera. Fiber number and fiber area of the soleus muscle were counted and measured using NIH Image software (NIH, Bethesda, MD).

Serum analysis. For the short-term experiment, paired serum samples were prepared for cytokine measurement. Preexercise blood samples were collected from the retroorbital plexus, and postexercise blood samples were collected from the inferior vena cava under anesthesia. Serum leptin and interleukin-6 (IL-6) concentrations were determined using a Mouse Serum Adipokine LINCOplex kit (Millipore, Billerica, MA) and Luminex 200 system (Luminex, Austin, TX).

Glycogen contents. Glycogen contents were determined in the short-term experiment. Liver and gastrocnemius muscle samples were frozen with liquid nitrogen, pulverized, and homogenized in ice-cold 0.3 M perchloric acid. Samples were incubated at room temperature for 2 h with amyloglucosidase (AG) reagent (50 nM NaAc, 0.02% BSA, AG, pH 5.5; Sigma, St. Louis, MO) and centrifuged for 10 min at room temperature. Glycogen concentrations were then determined by the hexokinase enzymatic method using glucose HK reagent (Sigma).

Fig. 1. Changes in daily (A) and cumulative (B) voluntary wheel-running distance during the long-term experiment (2-mo-old male mice, error bars = SD, n = 8 in each group). Both daily and cumulative distances of Snark<sup>−/−</sup>/ExL (exercised long term) mice were double those of Snark<sup>−/−</sup>/ExL (exercised) mice. *Significantly different (P < 0.05) from the corresponding values in Snark<sup>−/−</sup>/ExL mice.
RESULTS

Endurance capacity in the incremental treadmill running test. To assess the endurance capacity, mice underwent treadmill running until exhaustion (all-out test). Because sedentary Snark+/- mice exhibited less energy expenditure in our previous study (Tsucihara, unpublished observation), we assumed that the endurance capacity of Snark+/- mice should be impaired. Contrary to our expectations, running times until exhaustion were 31.82 ± 3.42 min in Snark+/- mice and 32.79 ± 3.24 min in Snark+/- mice; the difference was not significant.

Voluntary running activity. The most interesting finding in this study was that Snark+/- mice voluntarily ran longer distances than Snark+/- mice. At first, sedentary 3-mo-old female mice were placed in the wheel cage, and the daily running distance was monitored for 1 wk. Surprisingly, Snark+/- mice ran for longer than Snark+/- mice throughout the exercise period (mean daily distance: 5.88 ± 1.58 km in Snark+/- and 7.87 ± 1.47 km in Snark+/-; mean cumulative distance: 41.15 ± 11.27 km in Snark+/- and 55.07 ± 16.23 km in Snark+/-; P < 0.05); both daily and cumulative distances of Snark+/- mice were 1.3 times those of Snark+/- mice.

To further confirm the increased running distance, a long-term experiment was performed. Similar to female Snark+/- mice in the short-term experiment, male Snark+/- mice ran approximately two times the distance of Snark+/- mice daily and cumulatively over the 5-mo study period (mean daily distance: 5.64 ± 1.95 km in Snark+/- and 10.12 ± 1.80 km in Snark+/-; mean cumulative distance: 776.84 ± 154.34 km in Snark+/- and 1,493.36 ± 233.02 km in Snark+/-; P < 0.01; Fig. 1). Their running activities were pursuant to the 12:12-h light-dark cycles, although Snark+/-

Table 1. Body and tissue wet weights in male Snark+/- and Snark+/- mice in short- and long-term experiment (5 mo voluntary wheel running) and age-matched sedentary controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sedentary (control)</th>
<th>Exercise</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>+/-</td>
<td>+/+</td>
</tr>
<tr>
<td>Initial body wt, g</td>
<td>25.24 ± 0.70</td>
<td>25.39 ± 1.92</td>
<td>25.59 ± 2.62</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>33.54 ± 2.27</td>
<td>38.9 ± 2.04</td>
<td>30.18 ± 2.06</td>
</tr>
<tr>
<td>Heart, mg/g</td>
<td>4.64 ± 0.47</td>
<td>4.47 ± 0.56</td>
<td>4.56 ± 0.29</td>
</tr>
<tr>
<td>Liver, mg/g*</td>
<td>53.03 ± 4.09</td>
<td>52.73 ± 4.68</td>
<td>43.32 ± 5.47</td>
</tr>
<tr>
<td>Kidney, mg/g</td>
<td>6.28 ± 0.57</td>
<td>6.06 ± 0.60</td>
<td>6.51 ± 0.69</td>
</tr>
<tr>
<td>BAT, mg/g</td>
<td>4.86 ± 1.08</td>
<td>7.32 ± 1.58</td>
<td>2.68 ± 0.54</td>
</tr>
<tr>
<td>WAT, mg/g</td>
<td>20.68 ± 5.28</td>
<td>33.15 ± 6.74</td>
<td>11.95 ± 2.47</td>
</tr>
<tr>
<td>EDL, mg/g*</td>
<td>0.36 ± 0.05</td>
<td>0.31 ± 0.02</td>
<td>0.44 ± 0.11</td>
</tr>
<tr>
<td>TA, mg/g</td>
<td>1.86 ± 0.17</td>
<td>1.67 ± 0.13</td>
<td>1.88 ± 0.08</td>
</tr>
<tr>
<td>SOL, mg/g*</td>
<td>0.30 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>GAS, mg/g*</td>
<td>4.50 ± 0.24</td>
<td>4.26 ± 0.39</td>
<td>4.86 ± 0.28</td>
</tr>
<tr>
<td>PLA, mg/g*</td>
<td>0.57 ± 0.05</td>
<td>0.55 ± 0.06</td>
<td>0.69 ± 0.03</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 8 mice in each group. +/+, wild type; +/-, SNARK deficient; BAT, brown adipose tissue; WAT, white adipose tissue; EDL, extensor digitorum longus; TA, tibialis anterior; SOL, soleus; GAS, gastrocnemius; PLA, plantaris. *Main effect for condition (sedentary or exercise, P < 0.05). Significantly different (P < 0.05) from +/+ sedentary (†) and +/- sedentary (‡).
mice ran incessantly throughout the dark period of the cycle (Fig. 2).

**Body and tissue weights.** In the short-term experiment, no significant differences were found in body weight among the four groups at dissection (Snark<sup>+/+</sup>/CtlS: 22.43 ± 0.70 g; Snark<sup>+/+</sup>/ExS: 23.09 ± 0.89 g; Snark<sup>+/+</sup>/CtlL: 22.77 ± 0.66 g; Snark<sup>+/+</sup>/ExL: 22.01 ± 1.23 g; P = 0.60). WAT volumes of exercised mice were significantly less than those of sedentary mice in both genotypes (Snark<sup>+/+</sup>/CtlS: 8.89 ± 2.15 mg/g; Snark<sup>+/+</sup>/ExS: 5.38 ± 1.40 mg/g; Snark<sup>+/+</sup>/CtlL: 8.10 ± 2.30 mg/g; Snark<sup>+/+</sup>/ExL: 4.78 ± 0.90 mg/g; P < 0.05), whereas no significant differences were observed between genotypes. The weights of other tissues were not significantly different between groups.

After 5 mo of wheel running, the body weights of exercised mice were significantly lower than those of sedentary mice (P < 0.01, Table 1). This reduction was remarkably observed in Snark<sup>+/+</sup> mice. Note that body weights of sedentary mice were equivalent to those in our previous study (Snark<sup>+/+</sup>: 33.37 ± 1.12 g; Snark<sup>+/−</sup>: 41.34 ± 2.72 g; Tsuchihara, unpublished observation). Liver, BAT, and WAT weights were reduced in exercised mice, whereas the weights of most muscles were increased under exercise conditions. WAT weight was significantly heavier in Snark<sup>+/+</sup>/CtlL mice, although no significant differences were found between genotypes under exercise conditions (Fig. 3). WAT weights in both exercise groups (Snark<sup>+/+</sup>/ExL and Snark<sup>+/−</sup>/ExL) did not differ from those of preexercise controls (Snark<sup>+/+</sup>/CtlY and Snark<sup>+/−</sup>/CtlY).

**Body temperature and serum IL-6 level.** Basal body temperature of the mice in the short-term experiment was assessed. Snark<sup>+/−</sup> mice exhibited lower body temperature than Snark<sup>+/+</sup> mice in the preexercise period (P < 0.01). However, compared with the sedentary condition, body temperature in Snark<sup>+/−</sup>/ExS mice was elevated significantly during exercise (P < 0.05; Fig. 4) and reached a level equivalent to that in Snark<sup>+/+</sup> mice. The body temperature in Snark<sup>+/−</sup>/ExS then decreased in the postexercise period. After 2 wk of the postexercise period; however, the body temperature of Snark<sup>+/−</sup> mice returned to a level equivalent to that of Snark<sup>+/+</sup> mice by 3 days of exercise (from 35.9 ± 0.2 to 37.0 ± 0.4°C). No such dynamic changes in body temperature were observed in Snark<sup>+/−</sup> mice.

The level of the pyrogenic cytokine IL-6 was also dynamically altered by exercise. Serum IL-6 concentration was significantly lower in Snark<sup>+/+</sup>/CtlS than in Snark<sup>+/+</sup>/CtlS mice after the 3 days of exercise (Fig. 5A). However, the IL-6 level was about twofold higher in Snark<sup>+/−</sup>/ExS mice compared with Snark<sup>+/+</sup>/CtlS mice. Again, no significant difference was observed in the IL-6 level between Snark<sup>+/+</sup>/CtlS and Snark<sup>+/−</sup>/ExS mice.

**Food intake and serum leptin level.** In the short-term experiment, increased food intake was observed in Snark<sup>+/−</sup>/ExS mice (Fig. 6). Before starting the voluntary running (preexercise period), no significant difference was found in food intake between Snark<sup>+/+</sup> and Snark<sup>+/−</sup> mice. However, once the mice began to exercise (exercise period), Snark<sup>+/−</sup>/ExS mice showed an increase in food ingestion (P < 0.05), whereas no apparent increase was observed in Snark<sup>+/+</sup>/ExS mice. Furthermore, during the postexercise period, food intake of Snark<sup>+/−</sup>/ExS mice returned to the preexercise level. Consistent with the above findings, serum concentration of the anorexig pyrogenic cytokine leptin was decreased significantly in Snark<sup>+/−</sup>/ExS mice compared with Snark<sup>+/−</sup>/CtlS mice, whereas no significant changes were observed in Snark<sup>+/+</sup> mice (Fig. 5B). Similarly, daily food intake tended to be greater in Snark<sup>+/−</sup>/ExL than in Snark<sup>+/+</sup>/ExL mice throughout the long-term experiment (Snark<sup>+/+</sup>/ExL: 4.89 ± 0.61 g; Snark<sup>+/−</sup>/ExL: 5.44 ± 0.45 g; P = 0.06).

**Liver and muscle glycogen contents.** We assessed glycogen contents in the liver and gastrocnemius muscle in the short-term experiment (Fig. 7). Snark<sup>+/−</sup>/CtlS showed a high liver glycogen content, although the difference was not significant because of the large variances (P = 0.12). No significant differences were observed in muscle glycogen contents.

**Muscle fiber area, fiber type composition, and fiber number.** We also assessed muscle fiber area, fiber type composition, and fiber number in the soleus muscle of mice in the long-term exercise and sedentary groups (Fig. 8). Although no differences in muscle weight were found, the muscle fiber area in Snark<sup>+/+</sup> was smaller than that in Snark<sup>+/−</sup> mice (P < 0.05). When compared by condition, fiber areas of exercise groups were significantly larger than those of sedentary controls (P <
On the other hand, no significant differences in fiber type composition in the soleus muscle were observed between the genotypes (Snark/H11001/H11001 CtlL: 30.55 vs. 30.45%; Snark/H11001/H11002 CtlL: 31.2 vs. 30.96%; type I vs. type II), whereas a significant difference was found between sedentary and exercise conditions.

**DISCUSSION**

We observed less energy expenditure in sedentary Snark+/− mice, which was assumed to be responsible for the obesity of Snark+/− mice (Tsuchihara, unpublished observation). Impaired energy expenditure may induce impaired endurance capacity, as reported previously in IL-6-deficient mice as a mutant mouse model (6). Thus we assumed that the endurance capacity of Snark+/− mice was lower than that of wild-type controls. However, in contrast to our expectations, forced running capacity was not different between Snark+/− and Snark+/− mice.

The most interesting observations in the present study were provided by the voluntary wheel-running experiments in both short- and long-term experiments. Female 3-mo-old Snark+/− mice ran farther than Snark+/+ mice in the 1-wk short-term experiment; the distance run by Snark+/−/ExS mice was 1.3 times that of Snark+/+ExS mice. Similarly, male Snark+/−/ExL mice ran approximately two times the distance of Snark+/+ExL mice both daily and cumulatively over the 5-mo study period (2−7 mo of age). The increase in cumulative distance in the short-term experiment was the same as that in the first 1 wk of the long-term experiment, suggesting that increased voluntary running capacity did not depend on either sex or age. Liver glycogen contents were high in Snark+/− CtlS mice, although the difference was not significant. However, after 1 wk of wheel running, liver glycogen content decreased to the same level as in other groups. On the other hand, no changes in muscle glycogen content were observed. Thus increased wheel-running distance throughout the 5-mo experimental period in Snark+/− mice might not be influenced by either liver or muscle glycogen content. In both the short- and long-term experiments, running distances were within the

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**Fig. 4. Changes in body temperature in Snark+/− (A) and Snark+/− (B) mice during the short-term experiment (3-mo-old female mice, error bars = SD, n = 8 in each group). Colonic temperature was measured 6 h after the beginning of the light period. Significantly different (P < 0.05) from the corresponding values in Snark+/− Ctl mice.**

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**Fig. 5. Serum interleukin-6 (IL-6; A) and leptin (B) concentrations after the short-term experiment (3-mo-old female mice, after 3 days of voluntary wheel running, Error bars = SD, n = 5 in each group). IL-6 and leptin concentrations were measured as described in MATERIALS AND METHODS. Significantly different (P < 0.05) from Snark+/− CtlS (short-term control; †), Snark+/−/ExS (short-term exercised; ‡), and Snark+/−/ExS (§) mice.**
range reported previously in C57/BL6J mice (1, 23). Note that the results in Snark<sup>+/−</sup> and Snark<sup>+/+</sup> mice were in the higher and lower ends of the range of those in C57/BL6J mice, respectively. Although Snark<sup>+/−</sup> mice ran greater distances than their Snark<sup>+/+</sup> counterparts, their running activity was pursuant to the 12:12-h light-dark cycle, indicating that the greater running distance of Snark<sup>+/−</sup> mice was not the result of an aberrant circadian rhythm. Physical activity is known to be increased by anatomical and neurochemical impairment, leading to the disturbance of synchronization of the light-dark cycle under normal conditions. However, our results suggested that the greater running distance of Snark<sup>+/−</sup> mice was not because of these mechanisms.

Previous studies indicated that skeletal muscle metabolic adaptations to voluntary wheel running reached statistical significance 4 wk after the initiation of exercise in rodents (1, 2), but voluntary wheel running does not necessarily cause muscle hypertrophy (1, 15). In this study, the weights of most muscles were increased in the exercise condition. We also showed that muscle fiber area in the soleus muscle, which is a protagonist during exercise, was larger in the exercise condition, and thus 5 mo of voluntary running caused hypertrophy in both genotypes. The fiber area was smaller in Snark<sup>+/−</sup> than in Snark<sup>+/+</sup> mice under both conditions. Although Snark<sup>+/−</sup>ExL mice ran two times the distance of Snark<sup>+/−</sup>ExL mice, the difference between control and exercise conditions was slightly smaller in Snark<sup>+/−</sup> mice. Rodents that were allowed to run voluntarily on a wheel were shown to have reduced soleus cross-sectional areas compared with sedentary controls (29); hence, the increase in fiber area in Snark<sup>+/−</sup> mice might be attenuated by the greater running distance. Muscle fiber type composition is determined genetically, and dynamic changes in fiber type (e.g., type I fibers changing to type II fibers) are not easily caused by exercise (14, 29, 30, 33). In this study, however, we found significant differences in fiber type composition in the soleus muscle by condition, although no significant differences were observed between the Snark<sup>+/+</sup> and Snark<sup>+/−</sup> mice. Fiber number was also unaffected by genotype. Thus SNARK does not appear to have a direct effect on skeletal muscle composition.

The marked decrease in fat deposition in exercised Snark<sup>+/−</sup> mice also seemed to be attributable to their increased physical activity. Although body temperature of Snark<sup>+/−</sup> mice was lower than that of Snark<sup>+/+</sup> mice in the sedentary condition, it was elevated significantly within a few days after starting exercise and reached a level equivalent to that in Snark<sup>+/−</sup> mice. Elevated body temperature may reflect the increased energy expenditure and contribute to the consumption of deposited lipid in exercised Snark<sup>+/−</sup> mice.

Serum concentration of IL-6, which induces lipid mobilization and enhances lipid oxidation, was also significantly increased in Snark<sup>+/−</sup>ExS mice. IL-6 is a well-known proinflammatory cytokine (4), and thus an increased IL-6 level may contribute to the estimated body temperature of Snark<sup>+/−</sup>ExS mice. In contrast, serum IL-6 level and the basal body temperature of Snark<sup>+/−</sup>ExS mice were the same as those of Snark<sup>+/+</sup>CtlS mice. Several studies have shown that IL-6 levels are increased during exercise, as in the acute inflammatory response (27, 28). Kelly et al. (17) reported that IL-6 deficiency impairs exercise-induced AMPK activation in skeletal muscle, liver, and adipose tissue. It is plausible that AMPK activity is responsible for the IL-6-mediated increase in energy expenditure. Elevation of IL-6 after exercise is due to the working skeletal muscles (32). However, voluntary wheel running mostly involves low- to moderate-resistance exercise (19), and it may therefore be insufficient to stimulate an increase in serum IL-6 level and elevate body temperature in wild-type mice.

The mechanisms by which IL-6 production of Snark<sup>+/−</sup> mice was specifically induced during exercise should be discussed further. One possible explanation is that longer duration of running influenced muscle-derived IL-6 production even under low-intensity exercise. Another possibility is that SNARK has a role in regulating IL-6 production in skeletal muscles. Recently, Glund et al. (9) reported that AICAR, which activates AMPK and SNARK, suppressed IL-6 expression and release from isolated mouse skeletal muscles. Although the precise molecular mechanisms for this phenomenon have yet to be determined, it is possible that Snark deficiency diminishes the threshold for induction of IL-6 production in the skeletal muscles, and thus IL-6 level was increased even under voluntary running in Snark<sup>+/−</sup> mice. On the other hand, we observed that IL-6 level was significantly low in Snark<sup>+/−</sup>CtlS mice. In our previous study, IL-6 mRNA level was decreased in the liver but not in the skeletal muscles in...
intake was reduced in sedentary obese Snark\(^{+/−}\) mice (Tsuchihara, unpublished observation). Serum leptin concentration is positively correlated with body fat mass (8); therefore, we considered that the elevated leptin level in mature-onset obese Snark\(^{+/−}\) mice was related to a high volume of WAT. However, even though the WAT volume was not increased in younger Snark\(^{+/−}\) CtlS mice, elevated leptin was also observed in this study, suggesting that aberrant regulation of cytokine production may be preceded by apparent fat accumulation in Snark\(^{+/−}\) mice. In contrast to the elevation of IL-6, leptin level was reduced significantly in Snark\(^{+/−}\) ExS mice compared with

Fig. 7. Liver and gastrocnemius muscle glycogen contents in short-term experiment (3-mo-old female mice, after 3 days of voluntary wheel running, error bars = SD, \(n = 5\) in each group). Glycogen contents were measured as described in MATERIALS AND METHODS. No significant differences were observed.

Fig. 8. Histological results of soleus muscle in male Snark\(^{+/−}\) and Snark\(^{+/−}\) mice in the long-term experiment (error bars = SD, \(n = 5–8\) in each group). A: fiber area in the soleus muscle of Snark\(^{+/−}\) mice was significantly smaller than that of Snark\(^{+/−}\) mice (\(P < 0.05\)). After 5 mo of voluntary running, fiber area was increased significantly (\(P < 0.05\)). B: main effect of condition (sedentary or exercise) was observed on muscle type I fiber composition (\(P < 0.05\)), whereas no significant difference was found between genotypes.
Snark+/−/H11001 CtlS mice, whereas no similar changes were observed in Snark+/+ mice. Thus exercise may restore the aberrant regulatory mechanism that allows Snark+/−/H11001 mice to increase leptin production, although further investigations are required to determine the underlying mechanisms. The exercise-induced leptin reduction in Snark+/−/H11001 mice was consistent with the increased food intake in these animals with exercise.

Whether these extraordinary responses of cytokine production are simply a consequence of the increased voluntary running or actually account for the increased voluntary running is an interesting question for further studies. Leptin and other adipokines regulate feeding behavior through their actions in the hypothalamus (8). Decreased serum leptin concentration is one of the responses to starvation (8). On the other hand, it is well known that calorie restriction increases running and foraging activity in rodents (7, 13). Therefore, if the correlation between leptin level and starvation-induced increased movement could be confirmed, decreased leptin level may be a mechanism for the increases in voluntary running activity of Snark+/−/H11001 mice.

In conclusion, the results of the present study clearly indicated that Snark deficiency contributed to the regulation of physical activity. Our observations provide a basis for further studies to define a molecular mechanism of action of SNARK and to determine its physiological significance.

REFERENCES

