Exercise training restores decreased cellular immune functions in obese Zucker rats

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Moriguchi, S., M. Kato, K. Sakai, S. Yamamoto, and E. Shimizu. Exercise training restores decreased cellular immune functions in obese Zucker rats. J. Appl. Physiol. 84(1): 311–317, 1998.—This study investigated whether exercise training had a beneficial effect on the decreased mitogen response and improved a decreased expression of glucose transporter 1 (GLUT-1) in splenocytes from obese Zucker rats. Experimental groups were lean and sedentary and exercise-trained obese Zucker rats. Exercise training, running on a motor-driven treadmill for 5 days/wk for 40 wk, did not induce a significant decrease in body weight in obese Zucker rats. The plasma insulin concentration, showing a significant increase compared with lean Zucker rats, was unaffected by exercise training. However, the plasma triglyceride concentration in obese Zucker rats was significantly depressed by exercise training, whereas it was still higher than that in lean Zucker rats. In addition, natural killer cell activity and concanavalin A-induced mitogenesis of splenic lymphocytes of obese Zucker rats were significantly restored. In these splenic lymphocytes, glucose uptake was significantly lower compared with that in lean Zucker rats, which was also improved by exercise training. Although the expression of GLUT-1, the major glucose transporter in immune cells, was depressed in splenic lymphocytes of obese Zucker rats, exercise training induced a significant improvement. These results suggest that exercise training has a beneficial effect on the decreased cellular immune functions in obese Zucker rats, which is associated, in part, with the improvement in GLUT-1 expression.

obesity; mitogen response; glycogen uptake; glucose transporter; natural killer cell activity

METHODS

Experimental animals. Zucker rats were originally obtained from Kiwa Experimental Animal (Wakayama, Japan) and were propagated in our laboratory. At 8 wk of age female Zucker rats were divided into two groups: lean (n = 10) and obese (n = 20). Obese Zucker rats were further divided into two groups: sedentary control (n = 10) and exercised trained (n = 10). They were housed separately and were provided water and laboratory chow ad libitum after weaning. Until the end of the experiment (12 mo old), they were housed in a rodent facility at 22 ± 1°C with use of a 12:12-h light-dark cycle.

The training protocol consisted of running on a motor-driven treadmill (Yayoi, Tokyo, Japan) with an 8% grade, 5 days/wk for 40 wk. They ran during the lights-on portion of the light-dark cycle. Both treadmill speed and duration were increased rapidly such that by the third week of training the rats were capable of running three 15-min bouts at 15 m/min, separated by 5-min rest periods. This exercise intensity was selected because it has been found to improve the decreased mitogen response in old F344 rats (22). In the view of
circadian variations in lymphocyte proliferation (8), the rats were killed at 10:00 AM on day 3 after the last exercise bout. Blood collection and measurements of plasma glucose, insulin, and triglycerides. Blood was drawn from the inferior vena cava into heparinized syringes. Plasma obtained by centrifugation at 600 g for 20 min at 4°C was stored in aliquots at −40°C until glucose, insulin, and triglyceride concentrations were determined. Concentrations of glucose, insulin, or triglycerides in plasma were measured by using a B-Wako glucose test kit (Wako Pure Chemical Industries, Osaka, Japan), a double-antibody radioimmunoassay (Pharmacia, Uppskala, Sweden), and an E-Wako triglycerides test kit (Wako Pure Chemical Industries), respectively. Preparation and mitogenesis of splenocytes. Rats were anesthetized with pentobarbital sodium (0.1 ml/100 g body wt) and were exsanguinated by cutting off the arteries of both kidneys. The spleen was aseptically excised, weighed, and minced with scissors. The splenocytes were dissociated by using a stainless steel screen and adjusted to 1 × 10⁶ cells/ml using RPMI-1640 medium supplemented with 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 100 units/ml penicillin, 100 µg/ml streptomycin, and 50 µM 2-mercaptoethanol. Of each cell suspension, 0.1 ml was cultured for 72 h at 37°C with 0.1 ml of Con A (5.0 µg), a T-cell mitogen, and then they were immediately pulsed with 1.0 µCi [³H]thymidine (specific activity 25 µCi, New England Nuclear). Twenty-four hours later, the cells incorporated with [³H]thymidine were harvested onto a glass-fiber filter with a Mash I Harvester. Their radioactivity was counted by using a liquid scintillation counter after they had been kept overnight at room temperature. NK activity of splenocytes. NK-sensitive YAC-1 cells were used as target cells. A mixture of 0.05 ml ⁵¹Cr-labeled target cells (2 × 10⁵ cells/ml) and 0.1 ml of effector cells (2 × 10⁷ splenocytes/ml) was dispensed in U-bottomed microtiter plates. After centrifugation at 300 g for 5 min, the mixture was incubated for 4 h at 37°C in a 5% CO₂ incubator. Then, the plates were centrifuged again at 300 g for 5 min, and 0.1 ml of the supernatant was collected from each well. The radioactivity released from target cells was determined by using a gamma counter (ARC-363, Aloka, Tokyo, Japan). The percent lysis was calculated as follows:

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\text{%Lysis} = \frac{\text{experimental } ^{51} \text{Cr release} - \text{spontaneous } ^{51} \text{Cr release}}{\text{maximum } ^{51} \text{Cr release} - \text{spontaneous } ^{51} \text{Cr release}} \times 100
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Spontaneous ⁵¹Cr release was determined from target cells incubated with medium alone. Maximum ⁵¹Cr release was determined from target cells incubated with 0.1 M NaOH.

Glucose uptake by splenocytes. Splenocytes (1 × 10⁶ cells/ml) were cultured with Con A (5 µg/ml) for 48 h. Then, the supernatant of the culture was collected and used for the measurement of glucose as described above. Glucose uptake by splenocytes after in vitro incubation with Con A for 48 h was calculated by comparing the glucose concentration in the supernatant of cultured splenocytes from lean or obese Zucker rats with that of glucose in the supernatant without splenocytes.

Western immunoblotting for GLUT-1 protein. Cellular protein from splenic lymphocytes was extracted by lysing cells, which were cultured with Con A (5 µg/ml) for 48 h, in lysis buffer (phosphate-buffered saline containing 1% Triton X-100, 50 µg/ml aprogin, 10 µg/ml leupeptin, and 1 mmol/l phenylmethylsulfonyl fluoride), and quick-freezing the supernatant. The protein concentrations were assayed by using a DC Protein Assay Kit (Bio-Rad, Tokyo, Japan). Total protein (10 µg) was electroblotted onto nitrocellulose paper after separation on sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using 10–20% gradient gel. The filters were allowed to incubate for 2 h at 4°C with a 1:1,000 dilution of rabbit anti-rat GLUT-1 antibody purchased from East-Acrea Biologicals (Southbridge, MA). The immunoblot was subsequently incubated for 1 h with a 1:2,500 dilution of horseradish peroxidase-linked donkey anti-rabbit immunoglobulin antibody (Amersham, Bucks, UK). Positve bands were visualized by using the enhanced chemiluminescence detection system from Amersham. Expression of GLUT-1 in splenic lymphocytes was quantitated by using laser densitometer (Utrascan XL, Pharmacia).

Statistical analysis. Values are expressed as means ± SD. Statistical analyses were performed by using SPSS software (SPSS Japan, Tokyo, Japan), and statistical differences were tested by using the Mann-Whitney U-test. A level of \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Body and spleen weights and number of splenocytes. Obese Zucker rats had a significant increase in their body weight (833.0 ± 115.9 g), ~3 times greater than that in lean Zucker rats (256.0 ± 14.0 g). However, there were no significant differences in spleen weight per 100 g of body weight and number of splenocytes per gram spleen between lean and obese Zucker rats (1.45 ± 0.42 and 0.98 ± 0.26 g and 7.90 ± 1.09 × 10⁸ and 6.54 ± 0.85 × 10⁸ cells, respectively). As shown in Fig. 1, exercise training did not induce lower body weight in obese Zucker rats compared with sedentary obese Zucker rats.
Plasma glucose, insulin, and triglyceride concentrations. Although plasma insulin and triglyceride concentrations in obese Zucker rats were significantly higher than those in lean Zucker rats, there was no significant difference in that of plasma glucose (Fig. 2). Although exercise training did not have any effects on plasma glucose and insulin concentrations, plasma triglyceride concentration was significantly depressed by exercise training ($P < 0.05$).

Mitogen response and NK activity of splenic lymphocytes. Mitogenesis of splenic lymphocytes with use of Con A was measured by using the incorporation of $[^{3}H]$thymidine. A dose-response curve for Con A-stimulated proliferation was not shifted in obese rats compared with lean rats (Fig. 3). The concentration of Con A inducing maximum proliferation was 5 µg/ml in both lean and obese rats. The basal proliferation of unstimulated cells was lower in obese rats than that in lean rats (obese rats: $36.0 \pm 6.2$ counts/min, lean rats: $174.7 \pm 39.8$ counts/min). As shown in Fig. 4, mitogen response of splenic lymphocytes from obese Zucker rats was significantly lower than those from lean Zucker rats ($P < 0.01$). Exercise training improved the decreased mitogen response of splenic lymphocytes in obese Zucker rats until it was almost similar to that in lean Zucker rats. Although NK activity of splenocytes was also markedly depressed in obese Zucker rats compared with that in lean Zucker rats (Fig. 5), exercise training restored a decreased NK activity of splenocytes in obese Zucker rats to the level in lean Zucker rats.

Glucose uptake by splenocytes. Glucose uptake by splenocytes was measured after in vitro incubation with Con A (5.0 µg/ml) for 48 h. Without Con A stimulation, glucose uptake was not detected in splenocytes from either lean or obese rats. Glucose uptake by splenocytes from obese Zucker rats was significantly lower than by those from lean Zucker rats ($P < 0.05$), which was restored by exercise training (Fig. 6).

GLUT-1 expression in splenic lymphocytes. The main glucose transporter expressed on the membranes of lymphocytes and macrophages is GLUT-1 (21). The expression of GLUT-1 in splenic lymphocytes after in

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Fig. 2. Plasma glucose (A), insulin (B), and triglyceride concentrations (C) in lean, Sed obese, and trained obese Zucker rats. Values are means ± SD; $n = 10$ rats. In Sed and trained obese Zucker rats, values were significantly different from those in lean Zucker rats, *$P < 0.05$. In trained obese Zucker rats, values for triglyceride concentration were significantly different from those in Sed obese Zucker rats (#$P < 0.05$).

Fig. 3. Dose-response curve of concanavalin A (Con A)-stimulated proliferation. Splenocytes from lean (△), Sed obese (○), and trained obese (●) Zucker rats were incubated with various concentrations of Con A (0–25 µg/ml) for 72 h. Proliferation was then measured by uptake of $[^{3}H]$thymidine as described in METHODS. cpm, Counts/min. In Sed obese rats, values were significantly different from those in lean rats (**$P < 0.001$).

Fig. 4. Proliferation of splenic lymphocytes with use of Con A (5 µg/ml) in lean, Sed obese, and trained obese Zucker rats. Values are means ± SD; $n = 10$ rats. In Sed obese rats, values were significantly different from those in lean rats (**$P < 0.05$) and those in trained obese rats (#$P < 0.05$).
vitro incubation with Con A (5.0 µg/ml) for 48 h was measured by using Western blot analysis. In the case of unstimulated cells without Con A, GLUT-1 expression was not detected. The expression of GLUT-1 in splenic lymphocytes from obese Zucker rats was apparently lower compared with that in lean Zucker rats (Fig. 7A). In the case of measuring GLUT-1 expression in splenic lymphocytes by using a laser densitometer, GLUT-1 expression in obese Zucker rats revealed an ~80% reduction compared with that in lean Zucker rats. In addition, GLUT-1 expression in obese Zucker rats increased about twofold with exercise training (Fig. 7B).

DISCUSSION

Obesity has been linked to the occurrence of hyperlipidemia, diabetes mellitus, atherosclerosis, infections, and some types of cancer (10, 26). Some of these diseases appear to be associated with the decrease in cellular immunity after development of obesity. Although there are several reports showing that obesity induces the decrease in cellular immunity (20, 27, 28, 37), the mechanism remains to be elucidated. Zucker rats were used as an obese model in this study. Although plasma insulin and triglyceride concentrations were much higher in obese Zucker rats compared with those in lean Zucker rats (465% in insulin and 1,151% in triglycerides), plasma glucose concentration was unchanged. Despite hyperinsulinemia and hyperlipidemia, obese Zucker rats did not have diabetes mellitus. Because diabetes mellitus has a major impact on the immune system (5), Zucker rats are a suitable model to investigate the effects of obesity on cellular immunity.

As shown in Fig. 1, exercise training did not induce a significant decrease in the body weight of obese Zucker rats. This suggests that exercise training even without concomitant weight loss can induce beneficial changes in immune parameters in genetically obese rats. However, exercise training in this experiment significantly reduced the otherwise increased plasma triglycerides in obese Zucker rats, whereas plasma insulin was unaffected. Therefore, inducing improvement in insulin resistance may require more intense exercise (2). Because Nieman et al. (29) failed to show that improvements in the blood lipid and glucose profile were related to improvements in immune parameters after 12 wk of weight loss in obese humans, the beneficial effect of exercise training may be limited in obese Zucker rats and may not apply to humans.

Proliferation of splenic lymphocytes with use of Con A was significantly lower in obese rats than in lean Zucker rats, as shown in Fig. 3. This lower responsiveness of splenic lymphocytes from obese Zucker rats may be related, in part, to the increased insulin and triglyceride concentrations in plasma. The quality and quantity of dietary lipids modulate cellular immunity (13, 39). Increased plasma insulin is associated with the decrease in cellular immune functions such as NK activity and proliferation of peripheral blood lymphocytes (1, 33). Therefore, hyperinsulinemia and hyperlipidemia shown in obese Zucker rats may decrease cellular immunity. Prostaglandin E2 (PGE2) is regarded as a factor for inducing the decrease in cellular immunity after a high-fat or a high-polyunsaturated fatty acid diet (19). Decreased lymphocyte proliferation in obese Zucker rats may be due to the increased production of PGE2 from macrophages and/or lymphocytes. PGE2 is produced in large amounts by macrophages and lymphocytes in elderly people and animals (4, 15) and obese subjects (34). In obese Zucker rats the increased production of PGE2 might be induced, which resulted in inducing decreased proliferation of splenic lymphocytes with use of Con A. However, our previous results in this study did not support this hypothesis because decreased proliferation of splenic lymphocytes in obese Zucker rats was unaffected by in vitro addition of indomethacin, an inhibitor against cyclooxygenase as a key enzyme in the PGE2 synthetic pathway (unpublished observations). This suggests that the beneficial effect of exercise training on the decreased mitogen response in obese Zucker rats is not related to the action of PGE2. However, because exercise training decreased only triglyceride concentration in plasma (Fig. 1), improvement of hyperlipidemia by exercise
training may restore decreased cellular immune functions in obese Zucker rats. Further study is needed to clarify how the improvement of plasma triglyceride concentration after exercise training influences the decreased mitogen response in obese Zucker rats.

We have reported that the main energy source for mature lymphocytes is glucose, especially at the stage of proliferation after in vitro stimulation with mitogens (32). On the basis of this study, we postulated that the decreased lymphocyte proliferation in obese Zucker rats might be due to the impairment of glucose uptake by lymphocytes. Muscle glucose uptake is lower in obese Zucker rats compared with that in lean Zucker rats, which involves reduced plasma membrane GLUT-4 protein content and a defect in the insulin-stimulated activation of GLUT-4 (18, 35). However, there are no reports on glucose uptake of lymphocytes in obese Zucker rats. In our previous report, we found that glucose uptake by splenic lymphocytes was significantly lower in obese Zucker rats compared with that in lean Zucker rats. As shown in Fig. 6, glucose uptake by splenic lymphocytes was significantly lower in obese Zucker rats compared with that in lean Zucker rats, which was significantly improved by exercise training. This suggests that obese Zucker rats have an impaired glucose-transport system for splenic lymphocytes that improved with exercise training. Although several glucose transporters have been found in various tissues, GLUT-4, a major glucose transporter, is expressed in muscle and fat tissues and is closely associated with diabetes mellitus (11). In immune cells, GLUT-1 but not GLUT-4 is expressed on their membranes after mitogen stimulation (9), which is not dependent on insulin stimulation (25). Expression of GLUT-1 was remarkably depressed in splenic lymphocytes of obese Zucker rats compared with that in lean Zucker rats, which was largely improved by exercise training (Fig. 7). This suggests that decreased proliferation of splenic lymphocytes in obese Zucker rats is associated with the impairment of glucose uptake, which is due to the decreased expression of GLUT-1, and that exercise training improves a decreased mitogen response of splenic lymphocytes in obese Zucker rats through the improvement of GLUT-1 expression.

GLUT-1 expression depends on mitogen stimulation and the signal transduction after mitogen stimulation. If the number of mitogen receptors were decreased on cells from obese Zucker rats, this might result in the decreased expression of GLUT-1 on splenic lymphocyte membrane. However, there was no significant difference in the expression of mitogen receptors on splenic lymphocytes between lean and obese Zucker rats, as reported previously (24). Thus the decreased expression of GLUT-1 in splenic lymphocytes of obese Zucker rats.
GLUT-1 EXPRESSION AND MITOGEN RESPONSE IN OBESE RATS

It is well established that obesity is a risk factor for deteriorating cellular immune functions with aging (23). In this case, the decreased cellular immunity in elderly people (>60 yr old) may be due not only to the decreased responsiveness of immune cells but also to the decreased uptake of glucose through decreased expression of GLUT-1. In addition, exercise training has beneficial effects on diabetes mellitus via the improvement of GLUT-4 expression in muscle (7, 12). The question remains as to whether exercise training has a beneficial effect on GLUT-1 expression in immune cells and results in restoration of decreased cellular immunity in obese or elderly subjects. The present study indicates that exercise training has a beneficial effect on decreased cellular immune functions in obese Zucker rats, which is associated with an increased expression of GLUT-1 in splenic lymphocytes. However, it is still significantly less than the GLUT-1 protein level in the lean rats. On the basis of the much smaller percentage alteration (~20%) in glucose uptake of stimulated splenocytes shown in Fig. 6, it appears that only ~50% recovery of GLUT-1 protein found in splenocytes of obese Zucker rats is necessary to produce the level of glucose uptake measured in cells from the lean rats.

In conclusion, this study supports the hypothesis that the decreased lymphocyte mitogenesis in obese Zucker rats is involved in the decreased uptake of glucose by immune cells and decreased expression of GLUT-1. In addition, exercise training improves decreased NK activity and mitogen response of splenic lymphocytes in obese Zucker rats, which is associated with the improvement of GLUT-1 expression.

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Received 9 May 1997; accepted in final form 3 October 1997.

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